METHOD AND SYSTEM FOR DETECTING ANALYTES

FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to a method and system for detecting and/or identifying analytes. In particular, the present invention relates to a device utilizing electro mechanical technology for the purpose of, for example, determining toxicity of a fluid.

Much industrial and academic effort is presently directed at the development of integrated micro devices or systems combining electrical, mechanical and/or optical/electrooptical components, commonly known as Micro Electro Mechanical Systems (MEMS). MEMS are fabricated using integrated circuit batch processing techniques and can range in size from micrometers to millimeters. These systems can sense, control and actuate on the micro scale, and function individually or in arrays to generate effects on the macro scale.

The development of miniaturized devices for chemical analysis and for synthesis and fluid manipulation is motivated by the prospects of improved efficiency, reduced cost and enhanced accuracy. Efficient, reliable manufacturing processes are a critical requirement for the cost-effective, high-volume production of devices that are targeted at high-volume, high-throughput test markets.

In the most general form, MEMS consist of mechanical microstructures, microsensors, microactuators and electronics which are integrated into a single device or platform (*e.g.*, on a silicon chip). The microfabrication technology enables fabrication of large arrays of devices, which individually perform simple tasks but in combination can accomplish complicated functions.

One type of MEMS is a microfluidic device. Microfluidic devices include components such as channels, reservoirs, mixers, pumps, valves, chambers, cavities, reaction chambers, heaters, fluidic interconnects, diffusers, nozzles, and other microfluidic components. These microfluidic components typically have dimensions which range between several micrometers to several millimeters. The small dimensions of such components minimize the physical size, the power consumption, the response time and the waste of a microfluidic device as compared to other technologies.

In the area of life science, microfluidic devices are used in DNA chips, protein chips and total analysis systems (also known as lab-on-chip). The use of a microfluidic device in the fabrication process of a microchip facilitates the production of small and high-density spots on the substrate. Since only a small amount of solution is needed to make one chip, the cost of chip production is substantially reduced. In addition, a microfluidic device can created spots in consistent quantities and with uniform configurations, so as to enable highly accurate comparisons between spots.

Microfluidic devices are typically used in genetic, chemical, biochemical, pharmaceutical, biomedical, chromatography, medical, radiological and environmental applications. For example, in environmental applications, such devices are used for detecting hazardous materials or conditions, air or water pollutants, chemical agents, biological organisms or radiological conditions. The genetic and biochemical applications include testing and/or analysis of molecules, or reactions between such molecules in microfluidic devices.

In a microfluidic device, a plurality of determinations may be performed concurrently and/or consecutively. By having channels that have ultra small cross-sections, operations can be carried out with very small volumes. In addition, by having very sensitive detection systems, very low concentration of a detectable label can be employed. This allows for the use of very small samples and small amounts of reagents.

Droplet microfluidics refers to the set of technologies that are being developed for manipulating very small, substantially uniform, liquid drops, micro- to nano-liters in volume, which are supported on a solid surface, sandwiched between two solid plates or sucked into a solid channel. The manipulations include moving the droplets around, making them coalesce, and breaking them up. These technologies have a promising potential for developing commercially viable droplet-based microfluidic platforms for biotechnology and other applications. One of the reasons is that the smaller the length scale over which transport processes (convection, diffusion and reaction) take place, the faster the completion time of the process. As such, the drive toward high-throughput screening and diagnostics requires the concomitant development of associated microfluidic enabling technologies.

Droplet microfluidics may be employed in the area of biochemical and biophysical investigations of single cells. Knowledge of cell activity may also be gained by measuring and recording electrical potential changes occurring within a cell, which changes depend on the type of cells, age of the culture and external conditions such as temperature or chemical environment. Thus, precisely controlling the physical and chemical environment of a cell under study significantly enhances the value of the research. In addition, as further detailed hereinunder, cells activity can also exploited for the purpose of detecting and/or identifying chemicals in a sample.

The ability to sense, analyze, monitor and/or control transport of fluid through or from a microchannel is one of the fundamental properties required for all the above applications.

An objective in developing new techniques is not only to be able to selectively identify target compounds but to be able to assay large numbers of samples. Yet, there remain problems in detecting and measuring low levels of compounds conveniently, safely and quickly.

One of the favored approaches of analyzing, detecting and/or monitoring substances involves the use of light. Traditional methods involve illuminating a sample with light and using absorbance or scattering characteristics of the sample to analyze the analyte present therein. Typically, light based methods utilize one or more luminescent materials, such as fluorescent materials, either as labels or as reporters. Additionally, the analyte of interest can also have luminescent properties.

When a fluorescent atom or molecule absorbs light, electrons are boosted to a higher energy shell of an unstable excited state. During the lifetime of excited state (typically 1–10 nanoseconds) the fluorescent atom undergoes conformational changes and is also subject to a multitude of possible interactions with its molecular environment. The energy of excited state is partially dissipated, yielding a relaxed singlet excited state from which the excited electrons fall back to their stable ground state, emitting light of a specific wavelength. The emission spectrum is shifted towards a longer wavelength than its absorption spectrum. The difference in wavelength between the apex of the absorption and emission spectra of the fluorescent atom (also referred to as the Stokes shift), is typically small.

In a fluorescent material, not all the molecules initially excited by absorption return to the ground state by fluorescence emission. Other processes such as

collisional quenching, fluorescence resonance energy transfer and intersystem crossing may also depopulate the excited state. A ratio of the number of fluorescence photons emitted to the number of photons absorbed, called "fluorescence quantum yield," is a measure of the relative extent to which these processes occur. For fluorescent materials which are commercially available, only a small portion (about 0.1 %) of the absorbed light is actually emitted.

The low fluorescence quantum yield and the small separation between the absorption and emission spectra, require the usage of spectral discrimination methods to allow a clear detection. Typically, the discrimination methods utilize a set of filters on the excitation path and emission path of a fluorescence detection system. Such filters were greatly developed during the past years, and are being manufactured by various companies.

Heretofore, attempt to developed microfluidic devices for the purpose of detecting analysts, resulted in only partial success.

U.S. Patent No. 6,614,030 discloses an optical detection device for identifying and detecting fluorophores in during operations involving fluorescent signals. The optical detection device includes an excitation light source, and an optical setup for guiding excitation light and emission light. In use, the housing of the device is accurately moved over a small area in relation to a channel in a microfluidic device.

U.S. Patent No. 6,602,702 discloses a system for the rapid characterization of multi-analyte fluids. The system employs a sensor array, formed of a plurality of cavities, each trapping a chemically sensitive particle, which is configured to produce light when a receptor coupled to the particle interacts with an analyte of the fluid. The analytes within the fluid are then characterized by pattern recognition techniques.

U.S. Patent No. 6,551,838 discloses a device having a plurality of reservoirs covered by barrier layers which can be disintegrated or permeabilized to expose the isolated contents to the one or more environmental components. The reaction between the contents of the reservoirs and the environmental components generate a signal which is detected by conventional technique.

Several attempts have been made to incorporate biological materials as biosensors capable of sensing physical or chemical environmental conditions in microfluidic devices.

Generally, a biosensor is a device that qualifies and/or quantifies a physiological or biochemical signal. Biosensors have been developed to overcome some of the shortcomings of the classical analyte detection techniques. Good biosensing systems are characterized by specificity, sensitivity, reliability, portability, ability to function even in optically opaque solutions, real-time analysis and simplicity of operation. Biosensors couple a biological component with an electronic transducer and thus enable conversion of a biochemical signal into a quantifiable electrical response.

The function of the biosensor depends on the biochemical specificity of the biologically active material. Enzymes, antibodies, aptamers, DNA, receptors, organelles and microorganisms as well as plant cells or tissues have been used as biological sensing elements. The most commonly used biological element in the construction of biosensors are enzymes, due to their high specific activities as well as high analytical specificity. Purified enzymes are, however, expensive and unstable, thus limiting their applications in the field of biosensors.

The use of whole cells as the biosensing element negates the lengthy procedure of enzyme purifications, preserves the enzymes in their natural environment and protects it from inactivation by external toxicants such as heavy metals. Whole cells also provide a multipurpose catalyst especially when the process requires the participation of a number of enzymes in sequence. Whole cells have been used either in viable or non-viable form. Viable microbes, for example, can metabolize various organic compounds resulting in various end products like ammonia, carbon dioxide, acids and the like, which can be monitored using a variety of transducers [Burlage (1994) Annu. Rev. Microbiol. 48: 291-309; Riedel (1998) Anal. Lett. 31:1-12; Arikawa (1998) Mulchandani, Rogers (Eds.) Enzyme and Microbial Biosensors: Techniques and Protocols. Humanae Press, Totowa, NJ, pp.225-235; and Simonian (1998) Mulchandani, Rogers (Eds.) Enzyme and Microbial Biosensors: Techniques and Protocols. Humanae Press, Totowa, NJ pp:237-248].

The selection of microbial culture which corresponds well with a spectrum of compounds present in the sample is of significant importance.

A number of selection approaches are known in the art. For example, adaptation of a microbe for induction of desirable metabolic pathways and uptake systems can be effected by cultivation in a medium containing appropriate substrates

[Di Paolantonio and Rechnitz (1982) supra; Riedel (1990) Anal. Lett. 23:757-770; Fleschin (1998) Prep. Biochem. Biotechnol. 28:261-269]. Specifically, for the biochemical degradation of complex substrates such as mixtures of phenols, the use of activated sludge obtained from waste treatment plants can serve as an acclimatized mixed microbial consortium as compared to pure cultures [Joshi and D'souza (1999) J. Environ. Sci. Health Part A Eviron. Sci. Engng. 34:1689-1700].

Alternatively, when a single cell does not contain all enzymes necessary for a sequential set of reactions a mixture of microbial cultures can be used. Thus, Gluconobacter oxydans containing glucose oxidase has been used in conjunction with saccharomyces cerevisiae cells containing periplasmic invertase or permeabilized Kluyveromyces marxianus cells containing intracellular β-galactosidase, in the fabrication of a sucrose and a lactose biosensor, respectively [Svitel (1998) Biotechnol. Appl. Biochem. 27:153-158]. Note, the major drawback of such an approach is the need to maintain at least two cultures of microorganisms on a single sensor which may prove problematic such as due to different nutritional needs.

Microbial biosensors based on light emission from luminescent bacteria are also utilized in analyte detection. Bioluminescent bacteria are found in nature, their habitat ranging from marine to terrestrial environments. Bioluminescent whole cell biosensors have also been developed using genetically engineered microorganisms for the monitoring of organic, pesticide and heavy metal contamination. The microorganisms used in these biosensors are typically produced with an exogenous plasmid into which a reporter gene under the control of an inducible promoter of interest is placed.

Following are prior art technologies incorporating biosensors in microfluidic devices.

U.S. Patent No. 6,436,698 is directed at automatic measurement of water toxicity, using luminescent microorganisms living in freshwater. Test samples are injected using a needle into multi-well plate containing the luminescent microorganisms and, after a lapse of certain times from the injection, luminosity is detected by a sensor.

U.S. Patent No. 6,117,643 is directed at detection of pollutants, explosives and heavy-metals. A bioreporter, capable of metabolizing a particular substance to emit light, is placed in a selectively permeable container. When the light is emitted, an

optical application specific integrated circuit generates an electrical signal which indicates the concentration of the substance.

U.S. Patent No. 6,133,046 teaches the use of a fixed electrode and a moving electrode, whereby the surfaces of the electrodes bound a ligand of the analyte to be detected (*e.g.*, an antibody, whereby the analyte is an antigen or a hapten, a receptor whereby the analyte is a receptor, *etc.*). When a sample is placed between the electrodes, an electric signal is generated, depending on whether or not the analyte is present.

Additional prior art of relevance include: U.S. Pat. Nos. 6,638,752, 6,638,483, 6,636,752, 6,632,619, 6,627,433, 6,630,353, 6,620,625, 6,544,729, 6,537,498, 6,521,188, 6,453,928, 6,448,064, 6,340,572 and 5,922,537.

The above technologies suffer from many limitations. For example, in most prior art systems, the optical setup which is large, bulky and generally unsuitable for field use. In addition, there is the problem of obtaining a reliable optical signal, in effect compromising maximizing the signal from the detectable material while minimizing the background signal. Furthermore, in prior art systems which are based on mechanical scan (*e.g.*, moving electrode, moving light ray or moving sample), inaccurate readings may occur due to misalignment of the various components. With respect to the sensing process, it is difficult to generate transport of the sample in the channels and to distinguish between signals arriving from different locations.

There is thus a widely recognized need for, and it would be highly advantageous to have a method and system for identifying and/or detecting chemical agents and biological materials, devoid of the above limitations. The present invention provides solutions to the problems associated with prior art techniques aimed at multiplexed detection of a plurality of analytes.

SUMMARY OF THE INVENTION

According to one aspect of the present invention there is provided a device for detecting a presence of an analyte in a sample. The device comprising a device body configured with at least one reaction chamber configured for containing a sensor capable of producing a detectable signal when exposed to the analyte in the sample, the at least one reaction chamber being in fluid communication with at least one sample port and at least one actuator port via a first set of microfluidic channels

arranged such that application of a negative pressure to the at least one actuator port delivers fluid placed in the at least one sample port to the at least one reaction chamber.

According to further features in preferred embodiments of the invention described below, the at least one reaction chamber is configured so as to enable sustaining a negative pressure environment within the at least one reaction chamber.

According to still further features in the described preferred embodiments the at least one reaction chamber is configured such that the material placed therein does not substantially obstruct fluid flow in and out of the at least one reaction chamber.

According to still further features in the described preferred embodiments the microfluidic channels of the first set of microfluidic channels are connected to the at least one reaction chamber substantially above a bottom surface thereof.

According to still further features in the described preferred embodiments the at least one reaction chamber includes a plurality of reaction chambers sequentially interconnected via a second set of fluidic microchannels.

According to still further features in the described preferred embodiments the device further comprises a pumping device for generating the negative pressure.

According to still further features in the described preferred embodiments the device further comprises a sample reservoir being in fluid communication with the sample port.

According to still further features in the described preferred embodiments the device body is capable of allowing transmission of light having a predetermined wavelength therethrough.

According to still further features in the described preferred embodiments the device body comprises a material selected from the group consisting of silicon, plastic and glass.

According to still further features in the described preferred embodiments the device further comprises at least one humidity sensor, adapted for being positioned in the at least one reaction chamber, the humidity sensor being capable of generating a detectable signal when a level of humidity in the at least one reaction chamber is above a predetermined threshold.

According to still further features in the described preferred embodiments at least a portion of the plurality of chambers comprises different sensors, each capable

of generating a detectable signal when exposed to a different analyte of the at least one analyte.

According to another aspect of the present invention there is provided a device for detecting at least one analyte present in a sample, the device comprising at least one array of reaction chambers, each array having a plurality of reaction chambers, sequentially interconnected by a plurality of fluid channels, in a manner such that each reaction chamber is in direct fluid communication with at least two other reaction chambers, whereby a first reaction chamber of the at least two other reaction chambers serves as a fluid source and a second reaction chamber of the at least two other reaction chambers serves as a fluid sink, wherein each reaction chamber is designed for containing a sensor capable of generating a detectable signal when exposed to the at least one analyte.

According to further features in preferred embodiments of the invention described below, at least a portion of the sensors are biological sensors.

According to yet another aspect of the present invention there is provided a device for detecting at least one analyte present in a sample, the device comprising a substrate configured with: (a) a plurality of chambers for holding a fluorescent sensor and incubating reaction between the fluorescent sensor and the at least one analyte; (b) a plurality of fluid channels interconnecting at least a portion of the plurality of chambers; and (c) a plurality of waveguides designed and constructed to distribute excitation light among the plurality of chambers in a manner such that impingement of the excitation light on the fluorescent sensor is maximized and impingement of the excitation light on a surface of the substrate is minimized.

According to further features in preferred embodiments of the invention described below, the substrate is made of a disposable material.

According to still further features in the described preferred embodiments the plurality of waveguides are integrated with or formed in the substrate.

According to still further features in the described preferred embodiments the substrate is formed with a plurality of grooves, sizewise compatible with the plurality of waveguides, and further wherein at least a portion the plurality waveguides are designed insertable to and/or detachable of at least a portion of the plurality of grooves.

According to still further features in the described preferred embodiments the plurality of waveguides are arranged in a multi-furcated arrangement.

According to still further features in the described preferred embodiments the multi-furcated arrangement comprises a plurality of light splitting junctions, each capable of redirecting the excitation light into at least one of the plurality of waveguides.

According to still further features in the described preferred embodiments the plurality of waveguides are capable of imposing at least one predetermined propagation direction on the excitation light.

According to still further features in the described preferred embodiments the device further comprises at least one additional optical element, capable of imposing at least one predetermined propagation direction on the excitation light.

According to still further features in the described preferred embodiments the at least one predetermined propagation direction is substantially parallel to the surface of the substrate.

According to still further features in the described preferred embodiments the at least one additional optical element is selected from the group consisting of a diffraction grating, a reflection grating and a mini-prism.

According to still further features in the described preferred embodiments at least one of the plurality of chambers comprises a reflective coat, covering at least one internal wall of the chamber.

According to still further features in the described preferred embodiments the reflective coat is wavelength selective.

According to still further features in the described preferred embodiments the device further comprises a selective filter positioned on or close to the substrate and capable of prevention transmission of the excitation light therethrough.

According to still further features in the described preferred embodiments the device further comprises a plurality of optical focusing devices positioned so as to focus or collimate optical signals generated by the florescent sensor in response to the excitation light.

According to still further features in the described preferred embodiments the plurality of optical focusing devices are selected from the group consisting of microlenses and diffraction gratings.

According to still further features in the described preferred embodiments the fluid channels are microfluidic channels.

According to still further features in the described preferred embodiments the device further comprises a pump interface connectable to a pumping device.

According to still further features in the described preferred embodiments the device further comprises the pumping device.

According to still further features in the described preferred embodiments the device further comprises an electronic circuitry designed and constructed for controlling the plurality of micro-pumps.

According to still further features in the described preferred embodiments the plurality of fluid channels are connected to the plurality of reaction chamber substantially above a bottom surface thereof.

According to still another aspect of the present invention there is provided a device for detecting at least one analyte present in a sample, the device comprising a plurality of reaction chambers and a plurality of channels, interconnecting at least a portion of the plurality of reaction chambers, wherein each one of the plurality of reaction chambers comprises a biological sensor, capable of generating a detectable signal when exposed to the at least one analyte.

According to further features in preferred embodiments of the invention described below, the plurality of chambers are addressable, hence allow imaging thereof.

According to still further features in the described preferred embodiments at least a portion of the plurality of chambers comprises different biological sensors, each capable of generating a detectable signal when exposed to a different analyte of the at least one analyte.

According to still further features in the described preferred embodiments the plurality of reaction chambers are configured such that the biological sensor does not substantially obstruct fluid flow in and out of the plurality of reaction chambers.

According to still further features in the described preferred embodiments a body of the device is capable of allowing transmission of light having a predetermined wavelength therethrough.

According to an additional aspect of the present invention there is provided a system for detecting at least one analyte present in a sample, the system comprising: a

detecting device having a plurality of reaction chambers and a plurality of channels interconnecting at least a portion of the plurality of reaction chambers, wherein at least a portion of the plurality of reaction chambers comprises a sensor, capable of generating a detectable optical signal when exposed to the at least one analyte; a planar light detector capable of receiving optical signals from the detecting device and providing an image of sensors generating the optical signals.

According to further features in preferred embodiments of the invention described below, the system further comprises a data processor, supplemented by an algorithm for receiving image information from the planar light detector and determining presence of the at least one analyte.

According to still further features in the described preferred embodiments the system further comprises a control unit for sending control signals to the detecting device.

According to still further features in the described preferred embodiments the system further comprises a temperature control unit for controlling a temperature of the detecting device and/or the planar light detector.

According to still further features in the described preferred embodiments the algorithm is capable of determining concentration of the at least one analyte.

According to still further features in the described preferred embodiments the plurality of reaction chambers are configured so as to enable sustaining a negative pressure environment within the plurality of reaction chambers.

According to still further features in the described preferred embodiments the plurality of reaction chambers are configured such that the sensor does not substantially obstruct fluid flow in and out of the plurality of reaction chambers.

According to still further features in the described preferred embodiments the plurality of channels are connected to the plurality of reaction chamber substantially above a bottom surface thereof.

According to still further features in the described preferred embodiments at least a portion of the plurality of reaction chambers are sequentially interconnected via at least a portion of the channels.

According to still further features in the described preferred embodiments a body of the detecting device is capable of allowing transmission of light having a predetermined wavelength therethrough.

According to still further features in the described preferred embodiments the body comprises a material selected from the group consisting of silicon, plastic and glass.

According to still further features in the described preferred embodiments the sensor is a biological sensor.

According to still further features in the described preferred embodiments the planar light detector comprises a matrix having a plurality of addressable elementary units, each being capable of converting the optical signal into an electrical signal.

According to still further features in the described preferred embodiments the elementary units of the planar light detector are selected from the group consisting of positive-intrinsic-negative photodiodes, avalanche photodiodes, silicon chips and photomultipliers.

According to still further features in the described preferred embodiments the planar light detector is selected from the group consisting of a CCD camera and a CMOS detector.

According to still further features in the described preferred embodiments the system further comprises a light source for emitting excitation light so as to excite the sensor to thereby emit the optical signal.

According to still further features in the described preferred embodiments the light source comprises a light emitting diode.

According to still further features in the described preferred embodiments the light emitting diode is coupled to a collimator capable of redirecting the excitation light to form a substantially collimated light beam.

According to still further features in the described preferred embodiments the light source comprises an arrangement of light emitting diodes.

According to still further features in the described preferred embodiments each light emitting diode of the arrangement of light emitting diodes is coupled to a collimator capable of redirecting the excitation light to form a substantially collimated light beam.

According to still further features in the described preferred embodiments the system further comprises a temperature control unit for controlling a temperature of the detecting device, the planar light detector and/or the light source.

According to still further features in the described preferred embodiments the temperature control unit is selected from the group consisting of a thermoelectric device, a liquid cooler, a gas cooler and a blower.

According to still further features in the described preferred embodiments the system further comprises at least one selective filter positioned between the detecting device and the planar light detector, the at least one selective filter being capable of transmitting the optical signals and preventing transmission of the excitation light.

According to still further features in the described preferred embodiments the system further comprises a plurality of optical fibers for guiding the excitation light into the detecting device.

According to still further features in the described preferred embodiments the system further comprises an optical focusing device for focusing the optical signal on the planar light detector.

According to still further features in the described preferred embodiments the optical focusing device is a video lens.

According to still further features in the described preferred embodiments the system further comprises at least one opaque object, positioned between the detecting device and the planar light detector, wherein the optical focusing device is configured to focus the excitation light on the at least one opaque object thereby to substantially prevent impingement of the excitation light on the planar light detector.

According to still further features in the described preferred embodiments the system further comprises at least one reflector, positioned between the detecting device and the planar light detector, wherein the optical focusing device is configured to focus the excitation light on the at least one reflector so that the excitation light is reflected back to at least one of the plurality of reaction chambers.

According to still further features in the described preferred embodiments the system further comprises a transport mechanism for actuating transport of a sample fluid in the plurality of fluid channels, thereby to fill the plurality of reaction chambers with the sample fluid.

According to still further features in the described preferred embodiments the system further comprises a draining system and further wherein the transport mechanism is capable of maintaining a continues flow of the sample fluid in the

plurality of fluid channels thereby to continuously replace the sample fluid in the plurality of reaction chambers.

According to still further features in the described preferred embodiments the planar light detector is capable of providing the image substantially in real time.

According to still further features in the described preferred embodiments a portion of the plurality of reaction chambers comprises a material capable of generating a detectable reference optical signal at all times.

According to still further features in the described preferred embodiments the transport mechanism comprises a pumping device, capable of generating a negative pressure in the plurality of reaction chambers and the plurality of fluid channels.

According to still further features in the described preferred embodiments the pumping device comprises a plurality of micro-pumps.

According to still further features in the described preferred embodiments the system further comprises an electronic circuitry designed and constructed for controlling the plurality of micro-pumps.

According to still further features in the described preferred embodiments the electronic circuitry comprises at least one feedback line for monitoring operation and/or status of the plurality of micro-pumps.

According to still further features in the described preferred embodiments the transport mechanism further comprises a vacuum chamber connected to the pumping device and capable of maintaining a negative pressure environment.

According to still further features in the described preferred embodiments the transport mechanism further comprises a pressure sensor for sensing a pressure at an inlet of the vacuum chamber.

According to still further features in the described preferred embodiments the transport mechanism further comprises a flow sensor for sensing flow parameters of the sample fluid.

According to still further features in the described preferred embodiments the transport mechanism further comprises at least one tap for controlling the flow parameters.

According to still further features in the described preferred embodiments the transport mechanism further comprises at least one valve for activating and deactivating the transport of the sample fluid.

According to still further features in the described preferred embodiments the transport mechanism further comprises a hydrophobic filter for protecting at least one component of the transport mechanism.

According to still further features in the described preferred embodiments the system further comprises electronic circuitry for controlling flow rate of the sample fluid.

According to still further features in the described preferred embodiments the electronic circuitry is designed and constructed to allow equal filling of the sample fluid in the plurality of reaction chambers.

According to still further features in the described preferred embodiments the transport mechanism comprises an electric field generator, for generating a non-uniform electric field capable of inducing polarization on molecules of the sample fluid, hence to fill the plurality of reaction chambers with the sample fluid via dielectrophoresis.

According to still further features in the described preferred embodiments the transport mechanism comprises a column of the sample fluid, the column having a height selected such that a hydrostatic pressure, generated at a bottom of the column, is sufficient for actuating the transport of the sample fluid.

According to still further features in the described preferred embodiments the plurality of fluid channels are designed and constructed such that fluid sample flows therethrough via capillary forces.

According to still further features in the described preferred embodiments the sample is a liquid sample.

According to still further features in the described preferred embodiments the sample is a gas sample.

According to still further features in the described preferred embodiments the system further comprises a mechanism for binding components of the gas sample to an aqueous phase.

According to yet an additional aspect of the present invention there is provided an apparatus for imaging a pattern of optical signals received from a fluorescent material arranged in a plurality of predetermined locations, the apparatus comprising: (a) a planar light detector engaging a first plane; (b) a optical element engaging a second plane substantially parallel to the first plane; (c) a light source interposed

between the first and the second planes, the light source capable of generating excitation light in a direction other than a direction of the planar light detector; the optical element and the planar light detector being designed an constructed such that the excitation light is collimated by the optical element and impinges on at least a portion of the plurality of predetermined locations, and emission light, emitted by the fluorescent material in response to the excitation light, is focused by the optical element and impinges on the planar light detector, to form the pattern of the optical signal thereupon.

According to further features in preferred embodiments of the invention described below, the optical element comprises a plurality of lenses.

According to still further features in the described preferred embodiments the light source comprises a plurality of light emitting devices.

According to still further features in the described preferred embodiments an arrangement of the plurality of lenses is compatible with an arrangement of the plurality of predetermined locations.

According to still further features in the described preferred embodiments an arrangement of the plurality of light emitting devices is compatible with the arrangement of the plurality of lenses.

According to still further features in the described preferred embodiments the apparatus further comprises an infrared filter positioned between the planar light detector and the light source.

According to still further features in the described preferred embodiments the apparatus further comprises an additional optical element positioned between the light source and the planar light detector, the additional optical being capable of preventing the excitation light from impinging on the planar light detector.

According to still further features in the described preferred embodiments the additional optical element comprises at least one opaque object.

According to still further features in the described preferred embodiments the additional optical element comprises at least one reflector.

According to still further features in the described preferred embodiments a shape of the reflector is selected so as to direct the excitation light in a direction of the optical element engaging the first plane.

According to still further features in the described preferred embodiments each of the plurality of light emitting devices is positioned at a focal point of one lens of the plurality of lenses.

According to still an additional aspect of the present invention there is provided a method of determining concentration of an analyte from optical signals recorded of a reaction chamber in response to excitation light, the reaction chamber containing a plurality of biological sensors producing a fluorescent material when exposed to the analyte, the method comprising: (a) defining a plurality of slices, each slice having at least one biological reporter; (b) for each slice, representing the at least one biological reporter as at least one equivalent light emitter, located at a predetermined location within the slice, and calculating local radiation contribution emitted by the at least one equivalent light emitter; and (c) integrating the local radiation contribution over the plurality of slices so as to obtain an integrated radiation intensity; and (d) using the recorded optical signals and the integrated radiation intensity for determining the concentration of the analyte.

According to further features in preferred embodiments of the invention described below, the calculation of the local radiation contribution comprises calculating effective quantum efficiency and at least one transmission coefficient corresponding to the excitation light and light emitted by the at least one equivalent light emitter.

According to still further features in the described preferred embodiments the effective quantum efficiency comprises emission effective quantum efficiency and excitation effective quantum efficiency.

According to still further features in the described preferred embodiments the determination of the concentration of the analyte is by calculating an occupation area of the fluorescent material, the occupation area being defined as a projection of an occupation volume on a plane perpendicular to a direction of the excitation light.

According to a further aspect of the present invention there is provided a method of detecting analytes in a sample fluid, comprising: (a) providing a device having a plurality of reaction chambers and a plurality of channels, interconnecting at least a portion of the plurality of reaction chambers, wherein each one of the plurality of reaction chambers comprises a biological sensor, capable of generating a detectable signal when exposed to the at least one analyte; (b) filling at least a first portion of the

plurality of reaction chambers with the sample fluid; (c) generating a condition for the biological sensor to generate the detectable signal; and (d) detecting the detectable signal thereby detecting the analytes in the sample fluid.

According to further features in preferred embodiments of the invention described below, step (b) is by generating a negative pressure.

According to still further features in the described preferred embodiments step (b) is by dielectrophoresis.

According to still further features in the described preferred embodiments step (b) is by capillary transport.

According to still further features in the described preferred embodiments step (b) is by injection.

According to still further features in the described preferred embodiments the detectable signal is selected from the group consisting of a detectable optical signal, a detectable electrical signal and a detectable electrochemical signal.

According to still further features in the described preferred embodiments the biological sensors is capable of producing a bioluminescent material.

According to still further features in the described preferred embodiments the biological sensors is capable of producing a phosphorescent material.

According to still further features in the described preferred embodiments the biological sensor is capable producing a fluorescent material.

According to still further features in the described preferred embodiments step (c) comprises irradiating the biological sensor by excitation light.

According to still further features in the described preferred embodiments the method further comprises filling different portions of the plurality of reaction chambers with different fluids.

According to still further features in the described preferred embodiments at least one of the different fluids has a known composition, hence serving as a control fluid.

According to still further features in the described preferred embodiments different portions of the plurality of reaction chambers comprise different biological sensors.

According to still further features in the described preferred embodiments the method further comprises exposing each one of the biological sensors to at least two of the different fluids.

According to still further features in the described preferred embodiments the method further comprises generating an image of the reaction chambers.

According to still further features in the described preferred embodiments the method further comprises controlling a temperature of the detecting device.

According to still further features in the described preferred embodiments the controlling the temperature is by a thermoelectric device.

According to still further features in the described preferred embodiments the controlling the temperature is by a liquid cooler.

According to still further features in the described preferred embodiments the controlling the temperature is by a blower.

According to still further features in the described preferred embodiments the method further comprises using the detectable signal for determining a concentration of the analyte.

According to still further features in the described preferred embodiments the detecting device is disposable.

According to still further features in the described preferred embodiments step (d) is by a matrix having a plurality of addressable elementary units, each being capable of converting the optical signal into an electrical signal.

According to still further features in the described preferred embodiments the irradiation is by a light emitting diode.

According to still further features in the described preferred embodiments the method further comprises redirecting the excitation light to form a substantially collimated light beam.

According to still further features in the described preferred embodiments the irradiation is by an arrangement of light emitting diodes.

According to still further features in the described preferred embodiments the method further comprises a plurality of optical fibers for guiding the excitation light into the detecting device.

According to still further features in the described preferred embodiments the method further comprises focusing the optical signal prior to step (d).

According to still further features in the described preferred embodiments the optical focusing device comprises a plurality of lenses positioned to substantially prevent cross talks between different optical signals of different sensors.

According to still further features in the described preferred embodiments the method further comprises maintaining continues flow of the sample fluid in the plurality of fluid channels thereby to continuously replace the sample fluid in the plurality of reaction chambers.

According to still further features in the described preferred embodiments the method further comprises providing an image of the plurality of reaction chambers substantially in real time.

According to still further features in the described preferred embodiments a portion of the plurality of reaction chambers comprises a biological material capable of generating a detectable reference optical signal at all times.

According to still further features in the described preferred embodiments the method further comprises binding components of the gas to an aqueous phase.

According to yet a further aspect of the present invention there is provided a method of dehydrating a biological material, the method comprising: providing a first set of chambers for holding the biological material, and a second set of chambers having at least one fluid channel formed therein; placing the first set of chambers and the second set of chambers in a negative pressure environment so as to dehydrate the biological material; and positioning the second set of chambers on the first set of chambers so as seal the first set of chambers hence to maintain the negative pressure in the first and the second sets of chambers.

According to further features in preferred embodiments of the invention described below, each of the second set of chambers comprises a window for allowing evaporation of liquids therethrough.

According to still further features in the described preferred embodiments the method further comprises pressing the second set of chambers on the first set of chambers, such that when a respective chamber of the second set of chambers is pressed on a respective chamber of the first set of chambers a respective window is sealed.

According to still further features in the described preferred embodiments the pressing is done so as not to obstruct the at least one fluid channel.

According to still further features in the described preferred embodiments the method further comprises immobilizing the biological material to each of the first set of chambers, prior to the step of placing the first and the second sets of chambers in the negative pressure environment.

According to still further features in the described preferred embodiments the immobilizing is by encapsulating the biological sensor into a meltable membrane.

According to still further features in the described preferred embodiments the immobilizing is by encapsulating the biological material into a meltable membrane.

According to still further features in the described preferred embodiments the immobilizing is by a material selected from the group consisting of agar, alginate, poly-vinyl alcohol, sol-gel and carraginan.

According to still further features in the described preferred embodiments the biological sensors comprises a population of cells, the population of cells including a reporter expression construct being expressible in a cell of the population when exposed to the analyte.

According to still further features in the described preferred embodiments the population of cells is eukaryotic cells.

According to still further features in the described preferred embodiments the population of cells is prokaryotic cells.

According to still further features in the described preferred embodiments each of the reporter expression construct includes a cis-acting regulatory element being operably fused to a reporter gene.

According to still further features in the described preferred embodiments the reporter gene is selected from a group consisting of a fluorescent protein, an enzyme and an affinity tag.

According to still further features in the described preferred embodiments the cis-acting regulatory element is a promoter.

According to still further features in the described preferred embodiments the promoter is selected from the group consisting of *MipA, LacZ, GrpE, Fiu, MalPQ, oraA, nhoA, recA, otsAB* and *yciD.*

According to still further features in the described preferred embodiments the cis-acting regulatory element is stress regulated.

According to still further features in the described preferred embodiments the analyte is selected from the group consisting of a condition and a substance.

According to still further features in the described preferred embodiments the condition is selected from the group consisting of a temperature condition and a radiation condition.

According to still further features in the described preferred embodiments the substance is a naturally occurring product or a synthetic product.

According to still further features in the described preferred embodiments the populations of cells is tagged.

The present invention successfully addresses the shortcomings of the presently known configurations by providing a method, device and system for detecting and/or identifying analytes.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only, and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

In the drawings:

FIGs. 1a-b are schematic illustrations of a bottom view (Figure 1a) and a top view (Figure 1b) of a device for detecting one or more analytes, according to a preferred embodiment of the present invention;

FIG. 1c is a schematic illustration of a side view of one reaction chamber of the device of Figures 1a-b, according to a preferred embodiment of the present invention;

FIG. 2a-b are schematic illustrations of the reaction chambers and the fluid channels of the device in a preferred embodiment in which the chambers and the channels are arranged in one or more sequential arrays;

FIG. 3a is a schematic illustration of the device in the preferred embodiment in which a plurality of micro-pumps is employed;

FIG. 3b is a schematic illustration of one micro-pump, according to a preferred embodiment of the present invention;

FIG. 3c is a schematic diagram exemplifying a configuration of an electronic circuitry controlling the micro-pump of the device shown in Figure 3a, according to a preferred embodiment of the present invention;

FIG. 4 is a transport mechanism in a preferred embodiment in which external pumping is utilized, according to a preferred embodiment of the present invention;

FIG. 5 is a simplified illustration of a portion of the device in which the transport mechanism comprises an electric field generator, according to a preferred embodiment of the present invention;

FIG. 6 is a simplified illustration of a portion of the device in a preferred embodiment in which the transport of the sample fluid is generated by hydrostatic pressure;

FIG. 7 is a schematic illustration of a fluid channel in a preferred embodiment in which the sample fluid flows via capillary forces;

FIGs. 8a-c, are simplified illustrations of a system for detecting the analyte using optical signal, according to a preferred embodiment of the present invention;

FIG. 9 is a simplified illustration of a light detector, according to a preferred embodiment of the present invention;

FIGs. 10a-d are simplified illustrations of the device in a preferred embodiment in which the spatial separation of the excitation light from the optical signal is employed, using a plurality of waveguides;

FIG. 11a is a simplified illustration of a side view of a light source, in a preferred embodiment in which waveguides are employed;

FIG. 11b is a simplified illustration of a top view of a top view the light beam outputted by the light source, according to a preferred embodiment of the present invention;

FIG. 12 is a simplified illustration of system in a preferred embodiment in which the device is positioned between the light source and the light detector;

FIG. 13 is a schematic illustration of the system in a preferred embodiment in which a plurality of external optical fibers is employed;

FIG. 14 is a simplified illustration of the light path of the excitation light once entering the reaction chamber, according to a preferred embodiment of the present invention (Figure 14 is rotated anticlockwise by 90° relative to Figure 13);

FIG. 15 is a schematic illustration of a video-lens, which can be used as a focusing device, according to a preferred embodiment of the present invention;

FIGs. 16a-c are schematic illustrations of a light source of the system of Figure 13, according to a preferred embodiment of the present invention;

FIG. 17a is a simplified illustration of the light source in a preferred embodiment in which the light source is an arrangement of light emitting devices;

FIGs. 17b-c are simplified illustrations of the system in a preferred embodiment in which the light source of Figure 17a is positioned between the device and the light detector;

FIG. 18 is a flowchart diagram of a method of detecting analytes in a sample fluid, according to a preferred embodiment of the present invention;

FIG. 19 is a schematic illustration of a logical and physical division of the device, according to a preferred embodiment of the present invention;

FIGs. 20a-c are schematic illustration of method steps for dehydrating a biological material, according to a preferred embodiment of the present invention;

FIG. 21 is a flowchart diagram of a method of determining the concentration of the analyte from the optical signals, according to a preferred embodiment of the present invention;

FIGs. 22a-b are electronic diagrams of a CMOS detector, which can be used as a light detector, according to a preferred embodiment of the present invention;

FIG. 23a is a schematic illustration of the slicing technique of the method of Figure 21, according to a preferred embodiment of the present invention;

FIG. 23b is a schematic illustration of an equivalent light emitter which can be used in the slicing technique of the method of Figure 21, according to a preferred embodiment of the present invention;

FIG. 23c is a schematic illustration of the spreading of an optical signal through an aperture of the reaction chamber, according to a preferred embodiment of the present invention;

FIG. 24 is a schematic calculation diagram which can be implemented for the calculation of optical coefficients, according to a preferred embodiment of the present invention;

FIG. 25 illustrates light propagation from the equivalent light emitter to a lens, according to a preferred embodiment of the present invention;

FIG. 26 illustrates the scattering solid angle of the emitted light rays, according to a preferred embodiment of the present invention; and

FIGs. 27a-d show results of an experiment performed using the device of Figure 3a with fresh biological sensors.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is of a method, device and system for detecting and/or identifying analytes, which can be used for measuring fluid toxicity. Specifically, the present invention can be used to measure and analyze water or air toxicity using biological sensors, such as, but not limited to, bacteria. The present invention is further of an apparatus for reading data of a chip incorporating the biological sensors, and a method of determining the concentration the analytes based on the obtained data.

The principles and operation of a device, apparatus and system for detecting and/or identifying analytes according to the present invention may be better understood with reference to the drawings and accompanying descriptions.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details of construction and the arrangement of the components set forth in the following description, illustrated in the drawings or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways.

Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

According to one aspect of the present invention, there is provided a device for detecting one or more analytes, generally referred to herein as device **10**.

As used herein the term "analyte" refers to a molecule or a mixture of molecules in a liquid, gaseous or aerosol medium. It will be appreciated that molecules can be completely soluble in a liquid medium, alternatively they may be in a colloidal state. Thus analytes in liquid medium may be in solution or carried by the liquid medium.

Examples of analytes include, but are not limited to, small molecules such as naturally occurring compounds (*e.g.*, compounds derived from plant extracts, microbial broths, and the like) or synthetic compounds having molecular weights of less than about 10,000 daltons, preferably less than about 5,000 daltons, and most preferably less than about 1,500 daltons, electrolytes, metals, peptides, nucleotides, saccharides, fatty acids, steroids and the like.

As used herein the term "about" refers to ± 10 %.

Analytes typically include at least one functional group necessary for biological interactions (*e.g.*, amine group, carbonyl group, hydroxyl group, carboxyl group).

Referring now to the drawings, Figures 1a-b illustrate a bottom view (Figure 1a) and a top view (Figure 1b) of device **10**, according to a preferred embodiment of the present invention. In its simplest configuration, device **10** comprises a body or substrate **11**, a plurality of reaction chambers **12** and a plurality of channels **14**, interconnecting at least a portion of reaction chambers **12**. Reaction chambers **12** and channels **14** can be formed in or integrated with substrate **11** in any fabrication method known in the art. Device **10** is preferably disposable. Thus, substrate **11** preferably comprises a disposable material, such as, but not limited to, silicon, plastic and glass.

Figure 1c illustrates a side view of one of reaction chambers **12**. Reaction chamber **12** preferably comprises a sensor **18**, capable of generating a detectable signal when exposed to the at least one analyte. According to a preferred embodiment of the present invention any detectable signal can be generated by sensors **18**, depending on the type of sensor being utilized. Representative examples of detectable signals include, without limitation, optical, electrical and electrochemical signals. When the

detectable signals are optical, substrate **11** is preferably made transparent to the optical signals. Substrate **11** can also be manufactured so as to selectively allow transmission of light having a predetermined wavelength. This can be done, for example, by doping substrate **11** by an impurity, whereby the type and concentration of the impurity is selected in accordance with the wavelength of the optical signals.

Device **10** further comprises one or more sample ports **16**, being in fluid communication with chambers **12** via channels **14**. Sample ports **16** serve for feeding sample fluid **15** (gas or liquid) into channels **14** through which sample fluid **15** flows into reaction chambers **12** and interacts with the sensors. When sample fluid **15** is in gaseous state, its components (*e.g.*, organic components) are preferably bound to an aqueous phase prior to the feeding of sample port **16**.

Given that a sufficient number of sensors are utilized, the intensity of the signal, generated by population of sensors **18**, typically depends on the amount of detectable analyte being in contact therewith. For a given sample fluid having a given concentration, the intensity of the signal is proportional to the amount of sample fluid present in reaction chambers **12**. To optimize correlation between the intensity of the signal and the concentration of the analyte in sample fluid **15**, reaction chambers **12** are preferably of substantially equal volume. The advantage of this embodiment is that when chambers **12** are filled, the occupation ratio between the analyte and sensors **18** does not vary from one chamber to another, so that, once a functional relationship between the intensity of the signal and the concentration of analyte is established (*e.g.*, using a simple calibration curve, a mathematical model, *etc.*), the same functional relationship can be used for many reaction chambers.

Reaction chambers **12** are preferably addressable so as to allow imaging thereof, as further detailed hereinunder. In this embodiment, at least a few of reaction chambers **12** may comprise different sensors, each capable of generating the detectable signal when exposed to a different analyte.

Device **10** is typically of small dimensions. Preferably, the area of device **10** is less than about 10 cm^2, more preferably less than 1 cm^2, most preferably less than 0.1 cm^2. The number of reaction chambers and fluid channels of device **10** is not limited, and may vary from a few to a few hundreds of thousands of reaction chambers positioned on the same device. Thus, device **10** is preferably a microfluidic device so as to facilitate forming therein or integrating therewith a large number of reaction

chambers and fluid channels. When reaction chambers **12** comprise different sensors, device **10** is capable of providing a multiplexed detection of an enormous amount of different analytes.

Fluid channels **14** are preferably microfluidic channels. Transport of sample **15** from sample port **16** through channels **14** and into reaction chambers **12** can be effected using a variety of methods which are known in the art. Preferably sample transport is effected in a manner which enables provision of an equal fluid volume to each of reaction chambers **12**. This may be done by a judicious positioning of fluid channels **14** and reaction chambers **12**, as further detailed hereinunder.

Before providing a further detailed description of device **10**, as delineated hereinabove and in accordance with the present embodiment, attention will be given to the potential applications offered thereby.

Hence, device **10** can be employed in a variety of applications. For example, in the environmental field, device **10** can be employed to detect the presence of pollutants such as halogenated hydrocarbons (used as pesticides), polycyclic aromatic hydrocarbons (carcinogenic compounds), acrylamide, acrylic acid and acrylonitrile, organophosphorous compounds (used as pesticides, insecticides, and chemical warfare agents), nitroaromatic compounds, such as nitrophenols, picric acid, trinitrotoluene (used as xenobiotics present in wastes of chemical armament plants as in civil factories for dye, pesticide, and other chemical manufacturing).

Alternatively, device **10** can be employed in the food and fermentation industries, where there is a need for quick and specific analytical tools. Analysis is needed for monitoring nutritional parameters, food additives, food contaminants, microbial counts, shelf life assessment, compliance with specifications or regulations, and other olfactory properties like smell and odor.

In pharmaceuticals and medicine, device **10** can be used for drug identification and qualification (*e.g.*, determination of active ingredients in pharmaceutical formulations]. Device **10** can also be used for detecting narcotics and explosives such as trinitrotoluene (TNT), cyclonite (RDX), pentaerythritol tetranitrate (PETN) C-4 class explosives, and combinations thereof [Yinon, Y. and Zitrin, S. (1993) Modern Methods and Applications in Analysis of Explosives, John Wiley & Sons, Ltd., Sussex, U. K.].

Referring now again to the drawings, Figures 2a-b are schematic illustrations of reaction chambers **12** and fluid channels **14**, according to a preferred embodiment of the present invention. In an embodiment in which chambers **12** and channels **14** are arranged in one or more sequential arrays.

It is to be understood that the configurations shown in Figures 2a-b are not to be considered as limiting and that other arrangements of chambers **12** and channels **14**, such as, but not limited to, arrangement facilitating equal filling, are not excluded from the scope of the present invention.

Hence, each reaction chamber in an array (except the first and the last) is preferably in direct fluid communication with at least two other reaction chambers. For example, referring to Figure 2a, chamber **12a** is in direct fluid communication with chambers **12b** and **12c**, such that chamber **12a** is fed from chamber **12b** and drained through chamber **12c**. In other words, chamber **12b** serves as a fluid source for chamber **12a** and chamber **12c** serves as a fluid sink for chamber **12a**. One of ordinary skill in the art would appreciate that with such configuration, and an appropriate transport mechanism, all the reaction chambers in the array are equally filled. For an array of equal-volume reaction chambers, this embodiment ensures equal reaction conditions in all the chambers in the array. In the configuration shown in Figures 2a, chamber **12a** is connected via channel **14a** to a top surface **22** of chamber **12b** and via channel **14b** to a bottom surface **24** of chamber **12c**. However, this need not necessarily be the case, since, for some applications, it may be desired to feed and drain all chambers at the same height-level.

Figure 2b is a schematic illustration of an array of reaction chambers in a preferred embodiment in which chambers **12** are drained through channel **14a** (connected at top surface **22**) and fed through a channel **14c** connected at position **26** which is located above a bottom surface **24** of chambers **12**, between a lower part **29** and an upper part **31** thereof. Channels **14a** and **14c** are in fluid communication preferably via an additional fluid channel, designated **28** in Figure 2b. Channel **28** can be vertical or can have any orientation with respect to channels **14a** and **14c** provided the fluid communication therebetween is preserved. As shown in Figure 2b, in the presently preferred embodiment of the invention, sensors **18** are positioned at bottom surface **24**, below positioned **26** so that fluid flow is not substantially obstructed. Device **10** may further comprise an input buffer **30**, an output buffer **32** and/or an

actuator port **34**. Input buffer **30** is preferably in fluid communication with sample port **16** and serves as a fluid source for the first reaction chamber in the array. Output buffer **32** is preferably in fluid communication with actuator port **34** and serves as fluid sink for the last reaction chamber in the array. Actuator port **34** can be used to facilitate fluid transport as further detailed hereinbelow.

There are many techniques for actuating fluid transport in microchannels. Generally, a transport mechanism **62** is employed (see Figures 3a and 4, below). For example, in one embodiment transport mechanism **62** is capable of pumping or injecting sample fluid **15** through channels **14**. Mechanism **62** can be placed on or in device **10** or not, depending on considerations such as costs, size the like.

Several examples of micro-pumps or micro-injectors which can be utilized in mechanism **62** are known in the art. Mechanism **62** preferably enables sample **15** delivery by applying a negative pressure to actuator port **34**, channels **14** or reaction chambers **12**, thereby delivering sample **15** from sample port **16** to reaction chambers **12**.

As used herein "negative pressure" refers to a pressure value, which is smaller than a pressure value in a reference volume. For example, with respect to sample port **16**, "negative pressure" refers to a pressure value which is smaller than the pressure value in sample port **16**. The terms "negative pressure" and "under-pressure" are interchangeably used herein.

Reference is now made to Figure 3a which is a schematic illustration of device **10** in the embodiment in transport mechanism **62** comprises a plurality of micro-pumps **36** which are capable of generating a negative pressure in chambers **12** with respect to the pressure in sample port **16** (*i.e.* the pressure in chambers **12** is lower than the pressure in sample port **16**). According to a preferred embodiment of the present invention micro-pumps **36** are controlled by an electric circuitry (not shown, see Figure 3c), through a plurality of electrical contact, designated **38** in Figure 3a.

Figure 3b is a schematic illustration of one of micro-pumps **36**. Micro-pump **36** preferably comprises a substrate **42** (*e.g.*, glass substrate) onto which a layer **44** having a vacuum chamber **38** therein is applied. A puncturable membrane **40**, is deposited on vacuum chamber **46** thus buffering between vacuum chamber and an additional chamber **48** being in fluid communication with channel **14**. Membrane **40** can be made form any suitable materials, such as, but not limited to, silicon-nitride.

Additional chamber is sealed by a cover **50**, which is preferably, but not obligatory transparent.

When membrane **40** is punctured, the pressure in channels **14** drops thereby actuating flow of sample fluid from sample port **16** to reaction chamber **12**. The puncturing of membrane **40** is preferably by a heat shock which can be applied, for example, using a heater **43**, controlled by the electronic circuitry (not shown see Figure 3c) and positioned on or close to membrane **40**.

Reference is now made to Figure 3c, which is a schematic diagram exemplifying a preferred configuration of electronic circuitry **50** controlling micro-pump **36**. It is to be understood that the electric configuration of Figure 3c, as well as the accompanying description is not to be considered as limiting.

Hence, circuitry **50** can have Circuitry **50** includes a DC to DC switching converter **52** which is capable of charging a capacitor **54**, having a capacitance of, for example, 500 μF, to a predetermined voltage of, *e.g.*, 18 volts. A control line **58** may be connected to switching converter **52** for enabling or controlling the charging of capacitor **54**. Heater **43** can be connected to capacitor **54** through a Metal Oxide Semiconductor (MOS) transistor **56**. When a short regulated pulse is applied through line **59** to one of the gates transistor **56**, the gate opens and capacitor **54** is discharged through heater **43** thereby initiating the heat shock which punctures membrane **40** as further detailed hereinabove. A typical resistance of heater **43** is about 2 Ω, a typical activating current is about 9 A, and a typical a pulse duration is about 20 μs. Circuitry **50** may further comprise several feedback lines. One feedback line, designated **55** can be connected, *e.g.*, via an analog to digital converter **60**, to switching converter **52** and can be used for monitoring the status of capacitor **54**, another feedback line, designated **57** can be connected to transistor **56** for acquiring an activation status of heater **43**, hence to indirectly monitor whether or not membrane **40** is punctured.

Figure 4 is a schematic illustration of transport mechanism **62** in the embodiment in which external pumping is utilized. For illustrative purposes only, Figure 4 shows four pumping channels. It is to be understood that any number of pumping channels can be used.

According to a preferred embodiment of the present invention mechanism **62** comprises a pump interface **64** adapted to be connected to actuator port **34** or channels **14** and a vacuum chamber **74**, interposed between pump interface **64** and a pumping

device **76**, and being in fluid communication therewith. In operational mode, pumping device **76** reduces the pressure in vacuum chamber **74**, such that vacuum chamber **74** maintains a negative pressure environment. As a result, the pressure in interface **64** and actuator port **34** drops and actuates the flow of sample fluid **15** to reaction chamber **12**.

Mechanism **62** may further comprise a pressure sensor **72** for monitoring the pressure at the inlet of vacuum chamber **74**. Optionally and preferably, each pumping channel of mechanism **62** may further comprise a valve **63** capable of activating and deactivating the transport of sample fluid. Valve **63** is preferably a fast switching valve. A typical time delay of valve **63** is about 5 ms. The flow parameters (*e.g.*, speed, volume) of sample fluid **15** in each pumping channel is preferably monitored using a flow sensor **66** and regulated using a tap **68**. When sample fluid **15** is liquefied, the liquid may case damage to valve **63**. According to a preferred embodiment of the present invention, mechanism **62** preferably comprises a filter **70** made of a hydrophobic material which prevents sample fluid **15** from arriving to valve **63**. In the hydrophobic material of filter **70**, cohesive forces between like molecules dominate over external forces existing between the molecules of the liquid and molecules of filter **70**. The free surface of the liquid becomes film-like and the liquid is incapable of wetting filter **70** or penetrating therethrough.

The transport of sample fluid **15** may also be generated by electrical forces. When an uncharged particle (which may be, for example, a drop of sample fluid **15**) is placed in a non-uniform electric field, it becomes polarized, *i.e.*, acquires a non-zero electric dipole moment. The interaction between the electric dipole moment and the electric field results in net force acting on the fluid drop, which force is proportional to the electric dipole moment and the gradient of the electric field, and is commonly termed a dielectrophoretic force.

Reference is now made to Figure 5, which is a simplified illustration of a portion of device **10** in which mechanism **62** comprises an electric field generator **78**, according to a preferred embodiment of the present invention. Electric field generator **78** can be any device capable of generating a non-uniform electric field which induces polarization on molecules of sample fluid **15**. A representative example include, without limitation, two plates **80** of variable conductivity connected to a voltage source **82**. When a voltage is applied to plates **80**, dielectrophoretic forces generated

by the non-uniform electric field maneuver drop **84** of sample fluid **15** through the fluid channel.

The electric field is preferably designed and configured such that the dielectrophoretic forces direct drop **84** into reaction chambers **12**. Alternatively, the electric field is can be designed and configured such that the dielectrophoretic forces direct drop **84** away from reaction chambers **12**, for example, when it is desired to maneuver drop **84** from one chamber (*e.g.*, a filled or partially filled chamber) to an empty chamber (*e.g.*, an empty chamber). Still alternatively, the electric field is can be designed and configured such that the dielectrophoretic forces direct one portion of sample fluid **15** into reaction chambers **12** and another portion away from reaction chambers **12**, all depending on the desired filling of device **10**.

Reference is now made to Figure 6 which is a simplified illustration of a portion of device **10** in an embodiment in which the transport of sample fluid **15** is generated by hydrostatic pressure. Hence, in this embodiment mechanism **62** preferably comprises a column **86** of sample fluid **15** connected to sample port **16**. According to a preferred embodiment of the present invention the height of the column is selected such that the resulting hydrostatic pressure is sufficient for injecting sample fluid **15** into channels **14** and chambers **12**. Optionally and preferably, column **86** may be supplemented by a pressing device **88** (*e.g.*, a piston) for further increasing the pressure thereby to improve the flow of sample fluid **15** in channels **14**.

An additional transport technique which is contemplated is transport via capillary action. A capillary action is a phenomenon in which adhesion forces between molecules of the fluid and molecules of solid cause the fluid to flow through a small diameter channel. Hence, referring to Figure 7, according to a preferred embodiment of the present invention sample fluid **15** flows from sample port **16** into channels **14** via capillary forces generated between sample fluid **15** and walls **90** of channels **14**.

As stated, sensors **18** may generate optical signals when exposed to the analyte(s) in sample fluid **15**. This may be done by incorporating luminescent or fluorescent material in sensors **18**. The present invention successfully provides a system **20** for detecting the analyte using optical signal.

Reference is now made to Figures 8a-c, which are simplified illustrations of system **20**. In its simplest configuration system **20** comprises a detecting device, *e.g.*,

device **10**, and a light detector **108** for detecting an optical signal **106** generated by sensors **18** (not shown in Figures 8a-c). In the embodiment in which transport mechanism **62** is not an integral part of device **10**, system **20** may further comprise transport mechanism **62**. According to a preferred embodiment of the present invention system **20** further comprises a control unit **21** and a data processor **23**. Control unit **21** sends control signals to components of system **20**, for timing their operation. For example, control unit **21** may send activating and deactivating signals to light source **120** or mechanism **62**. Data processor **23** serves for processing signals received from detector **108** and thus is in data communication therewith.

System **20** may further comprise a power source **220** for supplying energy thereto. Power source **220** can be fixed or portable, replaceable or rechargeable, integrated with or being an accessory to system **20**. Examples of fixed power sources include, but are not limited too, a power source from a wall socket and a fixed voltage generator. Examples of a mobile power sources include, but are not limited too, an electrochemical cell (*e.g.*, a battery) and a mobile a voltage generator.

When power source **220** is portable, it can be implemented in device **10**, data processor **230**, light detector **108** or any other component of system **20**. In this embodiment, power source **220** can be, for example, a traditional secondary (rechargeable) battery, a double layer capacitor, an electrostatic capacitor, an electrochemical capacitor, a thin-film battery (*e.g.*, a lithium cell), a microscopic battery and the like. The type and size of power source **220** as well as the amount of energy stored therein may vary, depending on the required power and, in some embodiments, on the component in which power source **220** is implemented. For example, when data processor **230** is a portable computer, power source **220** can be an internal battery of the portable computer.

Detector **108** receives optical signal **106** from sensors **18** and converts signal **106** into electronic signals (*e.g.*, analog or preferably digital) which in turn can be received and analyzed, for example, by data processor **23**. Detector **108** preferably detects optical signals **106** simultaneously from several reaction chambers. More preferably, detector **106** detects optical signals **106** simultaneously from all the reaction chambers.

Data processor **23** is preferably designed to include software for determining the presence, absence or concentration of the analyte in sample fluid **15**. For example,

data processor **23** can determine whether or not sample fluid **15** is toxic and send an appropriate sensible signal to the user which can monitor the sensible signal, *e.g.*, using a display. Data processor **23** can also calculate the concentration of the analyte in sample fluid **15** and provide the user with the information desired.

Reference is now made to Figure 9, which is a simplified illustration of detector **108**, according to a preferred embodiment of the present invention. Detector **108** preferably comprises a matrix **105** having a plurality of addressable elementary units **107**, each being capable of converting light into electrical signal. Each elementary unit is allocated for a specific reaction chamber. When optical signal **106** originating from a particular reaction chamber impinges on matrix **105**, the respective elementary unit generates a signal, which can then be analyzed by data processor **23**. The signal generated by elementary units **107** preferably includes imagery information so as to allow attributing each signal to a respective reaction chamber, thereby providing an image thereof. Thus, according to a preferred embodiment of the present invention detector **108** is capable of providing an image of the sensors which generate optical signals **106**.

Several types of elementary detection units are contemplated herein. For example, elementary units **107** can be positive-intrinsic-negative (PIN) photodiodes. A PIN photodiode is a device having a large, neutrally doped intrinsic region sandwiched between p-doped and n-doped semiconducting regions. A PIN diode exhibits an increase in electrical conductivity as a function of the intensity, wavelength and modulation rate of incident radiation. The avalanche photodiode, is preferably used in accordance with the present invention since it is capable of generating an amplified current by avalanche multiplication in which electrons, initially generated by the incident light, accelerate and collide with other electrons.

Detector **108**, which incorporates PIN photodiodes or avalanche photodiodes enables accurate monitoring of intensity as well as the wavelength of optical signal **106**.

According to an alternative embodiment, detector **108** employs a charge-coupled device (CCD), in which elementary units **107** are silicon chips. When light hits the silicon chip, electrons are released from the crystalline structure of the silicon and deposited into small units or wells. Once the image is captured, the electrons in the wells are sent into a recorder where they are counted.

In another embodiment, detector **108** comprises at least one photomultiplier. Typically, a photomultiplier is a vacuum tube including a photocathode which is capable of converting light into electrons, by virtue of the photoelectric effect, an electron multiplier and an anode. When light enters the photocathode, the photocathode electrons are emitted into the vacuum and then directed by a system of focusing electrode towards the electron multiplier. The electron multiplier is a string of successive electron absorbers with enhanced secondary emission hence multiply the numbers of electrons. The amplification of the electron multiplier can reach eight orders of magnitude. Once multiplied, the electrons are collected by the anode as an output signal. Because of the high secondary-emission multiplication, the photomultiplier provides extremely high sensitivity and low noise.

According to yet another alternative embodiment, detector **108** employs complementary metal oxide semiconductor (CMOS) technology. The advantage of using the CMOS technology is that the elementary units and various quantification parts can be integrated into a single device, which may be compact and simple to operate. Such CMOS are commercially available such as for example the ACS-1394 fire-wire camera based on the ACS-1024 CMOS Image Sensor manufactured by Photonics Vision Systems, or IBIS4 CMOS Image Sensor manufactured by Fill Factory http://www.fillfactory.com). Further description of a CMOS imaging sensor which can be used as detector **108**, is provided in the Examples section which follows.

According to a preferred embodiment of the present invention system **20** further comprises at least one temperature control unit **25**, for controlling the temperature of system **20**. For example, temperature control unit **25**, can monitor and adjust the temperature of device **10**, detector **108** and/or light source **120** so as to optimize their operation. Temperature control unit **25** can be, for example, a thermoelectric device.

A thermoelectric device is a device that either converts heat directly into electricity or transform electrical energy into pumped thermal power for heating or cooling. Such a device is based on thermoelectric effects involving relations between the flow of heat and electricity through solid bodies. Generally, a thermoelectric device comprises at least one pair of dissimilar metals. When the device is used for cooling or heating, a potential difference is applied on the dissimilar metals and heat is pumped from one metal to the other. When the device is used for converting heat to

electricity (*e.g.*, for the purpose of monitoring the temperature of an object relative to a reference environment), the two metals are kept at different temperatures, and a potential difference is produced across.

Other temperature control units include, but are not limited to, liquid coolers, gas coolers, blowers and the like.

When sensors **18** include fluorescent material, optical signal **106** is generated in response to an excitation light **100**, emitted by light source **120**. Several configurations for positioning light source **120** are contemplated, depending on the relative angle between the detected portion of optical signal **106** and excitation light **100**. For example, in one embodiment, illustrated in Figure 8a, light source **120** is positioned on the side of device **10**, such that the detected portion of optical signal **106** is substantially perpendicular to excitation light **100**. In another embodiment, illustrated in Figure 8b, light source **120** is positioned above or below device **10** in a manner such that device **10** is between light source **120** and light detector **108**. In this embodiment, the detected portion of optical signal **106** is substantially parallel to excitation light **100**. In an alternative embodiment, illustrated in Figure 8c, light source **120** is positioned between device **10** and light detector **108**. In this embodiment, the detected portion of optical signal **106** is substantially anti-parallel to excitation light **100**. These embodiments are further detailed and exemplified hereinafter.

It is to be understood that, although Figures 8a-c show light detector **108** positioned below device **10**, this configuration is not to be considered as limiting.

The low fluorescence quantum yield of presently available fluorescent materials requires a separation between the optical signal and the excitation light. According to a preferred embodiment of the present invention the separation of the excitation light from the optical signal can be spatial separation and/or in the spectral separation.

As used herein, the term "spatial separation" refers to confinement of light energy to propagate only in a predetermined volume, irrespective of its wavelength, and the term "spectral separation" refers to absorption or reflection of certain wavelengths and transmission of other wavelengths.

Reference is now made to Figure 10a-d, which are simplified illustrations of device **10** in an embodiment in which spatial separation of the excitation light from the

optical signal is employed. This embodiment is useful when the configuration of Figure 8a is employed. Hence, according to the presently preferred embodiment of the invention device **10** comprises a plurality of waveguides **92** distributing excitation light **100** among chambers **12**.

As the present embodiment relies upon the ability to transmit and emit light through a waveguide, a brief description of such technology is provided hereinbelow.

The technology to transmit and guide light rays through optical systems exploits a physical phenomenon known as total internal reflection, in which a light is confined within a material surrounded by other materials with lower refractive index. When a ray of light moves within a transparent substrate and strikes one of its internal surfaces at a certain angle, the ray of light can be either reflected from the surface or refracted out of the surface into the open air in contact with the substrate. The condition according to which the light is reflected or refracted is determined by Snell's law, which is a mathematical relation between the impinging angle, the refracting angle (in case in case of refraction) and the refractive indices of both the substrate and the air. Broadly speaking, depending on the wavelength of the light, for a sufficiently large impinging angle (also known as the critical angle) no refraction can occur and the energy of the light is trapped within the substrate. In other words, the light is reflected from the internal surface as if from a mirror. Under these conditions, total internal reflection is said to take place.

Many optical devices operate according to the total internal reflection phenomenon. One such optical device is the optical fiber. Optical fibers are transparent flexible rods of glass or plastic, basically composed of a core and cladding. The core is the inner part of the fiber, through which light is guided, while the cladding surrounds it completely. The refractive index of the core is higher than that of the cladding, so that light in the core impinging the boundary with the cladding at a critical angle is confined in the core by total internal reflection.

As stated, total internal reflection occurs only for light rays impinging the internal surface of the optical fiber with an angle which is larger than the critical angle. Thus, a calculation performed according to geometrical optics may provide the largest angle which is allowed for total internal reflection to take place. An important parameter of every optical fiber (or any other light transmitting optical device) is known as the "numerical aperture," which is defined as the sine of the largest incident

light ray angle that is successfully transmitted through the optical fiber, multiplied by the index of refraction of the medium from which the light ray enters the optical fiber.

Another optical device designed for guiding light is the graded-index optical fiber, in which the light ray is guided by refraction rather than by total internal reflection. In this optical fiber, the refractive index decreases gradually from the center outwards along the radial direction, and finally drops to the same value as the cladding at the edge of the core. As the refractive index does not change abruptly at the boundary between the core and the cladding, there is no total internal reflection. However, although no total internal reflection takes place, the refraction bends the guided light rays back into the center of the core while the light passes through layers with lower refractive indexes.

Optical fibers are available in various lengths and core-diameters. For large core diameters, glass optical fibers are known to be more brittle and fragile than plastic optical fiber.

Another type of optical device is based on photonic materials, where the light ray is confined within a band gap material surrounding the light ray. In this type of optical device, also known as a photonic material waveguide, the light is confined in the vicinity of low-index region. One example of a photonic material waveguide is a silica fiber having an array of small air holes throughout its length. This configuration is capable of providing lossless light transmitting, *e.g.*, in either cylindrical or planar type waveguides.

Thus, according to a preferred embodiment of the present invention, each of waveguides **92** can be an optical fiber, a graded-index optical fiber a photonic material or any other optical device capable of transmitting light.

It is expected that during the life of this patent many relevant technologies for guiding light will be developed and the scope of the term waveguide is intended to include all such new technologies *a priori*.

Irrespectively of their type and operation principle, waveguides **92** can be integrated with or formed in body or substrate **11** of device **10**. Alternatively, device **10** may be manufactured with a plurality of grooves **94** (see Figure 10b) sizewise compatible with waveguides **92** such that waveguides **92** are inserted into grooves **94** prior to the excitation procedure. This embodiment is particularly useful when device

10 is made of a disposable material and it is desired to keep waveguides **92** for additional uses once device **10** is discarded.

As shown in Figure 10a, waveguides **92** are preferably arranged in a multi-furcated arrangement ("a tree"), having a plurality of light splitting junctions **98**, such that excitation light **100** enters through a single primary waveguide, designated **92a**, and distributed by light splitting junctions **98** to secondary waveguides, designated **92b** and **92c**. Each light splitting junction **98** is preferably designed to satisfy the numerical apertures of its outgoing waveguides. Waveguides **92** may also be arranged in several multi-furcated trees, so that excitation light **100** can enter device **10** through several primary waveguides. This embodiment is useful when several excitation wavelengths are used, whereby each multi-furcated tree of waveguides is dedicated to a particular excitation wavelength.

In any event, according to a preferred embodiment of the present invention waveguides **92** distribute the light in a manner such that impingement of the excitation light on sensors **18** is maximized and impingement of excitation light **100** on a surface **96** of substrate **11** is minimized. Once the fluorescent material in sensors **18** is excited by light **100**, an optical signal **106** is generated and can be detected, for example, using a light detector **108** position in the light path of signal **106**, as further detailed hereinafter.

The minimization of impingement on surface **96** and the maximization of impingement on sensors **18** can be better understood from Figures 10c-d which illustrate a side view (Figure 10c) and a top view (Figure 10d) of one waveguide **92** guiding light **100** into chamber **12**.

Referring to Figure 10c, the minimization of the impingement of excitation light **100** on surface **96** can be achieved by imposing a predetermined propagation direction on light **100**. More specifically, according to a preferred embodiment of the present invention, when exiting waveguide **92** and entering chamber **12**, light **100** propagates a direction which is substantially parallel to surface **96**.

Referring to Figure 10d, the maximization of impingement of light **100** on sensors **18** can be achieved by allowing light **100** to exit waveguide in a plurality of co-planar direction, each being parallel to surface **96**. A predetermined propagation direction can be imposed on light **100** either directly by waveguide **92** or by one or more additional optical elements **104**, *e.g.*, a diffraction grating, a reflection grating, a

mini-prism and the like. Optionally and preferably chamber **12** my comprise a reflective coat **102**, covering the walls of chamber **12**, so as to reflect light **100** hence to further increase the impingement of light **100** of sensors **18**. As further detailed hereinunder and in the Examples section that follows, sensors **18** may be biological sensors. In this embodiments coat **102** is preferably made of a biocompatible material.

Thus, excitation light **100** is constrained to propagate in a predetermined direction while an optical signal **106**, generated by sensors **18** in response to light **100**, is allowed to propagate in all directions. According to a preferred embodiment of the present invention at least one side of chamber **12** (*e.g.*, a bottom side **107**) is not coated by coat **102**. One of ordinary skill in the art would appreciate that with such configuration, optical signal **106** can be detected by detector **108** without being screened by excitation light **100** which is substantially confined in chamber **12**.

It is appreciated, however, that light **100** can be diverted, for example, when light **100** is not absorbed by sensors **18** but rather being scattered to a different direction. Thus, according to a preferred embodiment of the present invention, the aforementioned spatial separation is combined with spectral separation. For example, an emission filter **110** can be positioned in the light path of optical signal **106** so as to prevent diverted rays of excitation light **100** from arriving to detector **108**. Emission filter **110** preferably allows transmission of optical signal **106** substantially without loses. Additionally or alternatively, coat **102** can be a selective coat, capable of selectively reflecting light of a particular wavelength. In this embodiment, coat **102** may cover also bottom side **107** of chamber **12** so that optical signal **106** is transmitted therethrough and light **100** is reflected thereby.

According to a preferred embodiment of the present invention, optical signals generated in different reaction chambers are spatially separated so as to prevent cross talks between the different optical signals. This can be done, for example, by positioning an optical focusing device **112** (*e.g.*, a microlens) in the light path of optical signal **106** so as to focus signal **106** on detector **108**. Alternatively device **112** can be positioned so as to collimate signal **106** to a predetermined direction. In this embodiment, the optical signals of different chambers are preferably collimated to propagate in parallel directions thereby preventing cross talks therebetween. Optionally, a plurality of optical separations **114** can be positioned between different optical signals so as confined each optical signal not to cross the light path of the other

optical signals. Optical separation **114** can be, for example, a reflector to minimize losses.

Reference is now made to Figure 11a, which is a simplified illustration of a side view of light source **120**, in the embodiment in which waveguides **92** are employed. According to a preferred embodiment of the present invention light source **120** comprises a light emitting device **122**, a collimator **124** and a light coupler **127**. Light emitting device **122** can be, for example, a light emitting diode (LED), covered by a collimating lens **126**, capable of partially collimating the light emitted by the LED. Typically, lens **126** has a diameter of about 5 millimeters and is capable of providing a beam having a divergence of about 15°. Collimator **124** serves for further collimating light **100** and light coupler **127** serves for reducing the diameter of the light beam so as to facilitate coupling of light **100** into waveguide **92**. Light coupler **127** can be any known device for coupling a light into a waveguide in a manner that the impinging angle of the light on the waveguide satisfies its numerical aperture. One such light coupler which is commercially available is known as a "pigtail."

Figure 11b, schematically illustrate a top view the light beam outputted by light source **120**. As shown in Figure 11b, the light beam propagate in a plurality of co-planar direction thus maximizing the impingement of light **100** on sensors **18** as further detailed hereinabove.

Typically but not obligatory, light emitting device **122** emits blue light (*e.g.*, wavelength of about 470 nm) at an optical power of about 5 mW. Typical dimensions of light emitting device **122** are about 5 mm in width and about 10 mm in length.

Reference is now made to Figure 12 which is a simplified illustration of system **20** in an embodiment in which device **10** is positioned between light source **120** and detector **108**. Thus, in this embodiment, the detected portion of optical signal **106** is substantially parallel to excitation light **100**.

It is recognized that when optical signal **106** is parallel to excitation light **100**, a spectral separation between optical signal **106** and excitation light **100** is required. Thus, system **20** preferably comprises one or more selective filters for selectively allowing transmission of light having predetermined wavelength. One such selective filter is preferably an excitation filter **130** which allows transmission of excitation light **100** and substantially prevents transmission of light having different wavelengths. Another such selective filter is the aforementioned emission filter **110** which allows

transmission of optical signal **106** and substantially prevents transmission of light having different wavelengths.

To facilitate substantially simultaneous excitation of the sensors in all or at least a portion of chambers **12**, light source **120** preferably comprises a plurality of light emitting devices, which can be, for example, similar to the aforementioned light emitting device **122**. Optionally and preferably, system **20** comprises one or more separators **132** for substantially preventing cross talks between different excitation light rays.

To provide a better separation between excitation light rays emitted by different light emitting devices, system **20** can employ an arrangement of optical fibers, as further detailed hereinbelow.

Reference is now made to Figure 13, which is a schematic illustration of system **20** in an embodiment in which a plurality of external optical fibers are employed. Generally, in this embodiment system **20** comprises a first housing **138** holding devices **122** and excitation filter **130**, a second housing **142** for holding device **10** and a third housing **146** for holding detector **108** and emission filter **110**. First hosing **138** is preferably connected to second housing **142** by supporting legs **152**, and second housing **142** is preferably connected to third housing by supporting legs **154**.

According to a preferred embodiment of the present invention system **20** comprises a plurality of optical fibers **134** which deliver excitation light **100** from emitting devices **122** to device **10**. Preferably, as shown in Figure 13, each optical fiber delivers excitation light **100** to one of chambers **12**. Alternatively, one optical fiber can deliver light **100** to more than one chamber. A first end of each of optical fibers **134** is preferably connected via a groove **136** to housing **138** and a second end thereof is preferably connected, via a groove **140** to second housing **142**.

First housing **138** and second housing **142** are preferably made of a thermally conductive material, so as to allow temperature control unit **25** to monitor and control the temperatures thereof. Optionally, system **20** can comprise one or more thermistors being in thermal communication with first **138** and/or second **142** housings, for sensing the temperatures.

Second housing **142** is preferably thermally isolated from detector **108**. This can be done, for example, using one or more thermal isolators **144** positioned adjacently to second housing **142** and/or third housing **146**.

Optionally an additional filter, an infrared filter **148**, can be positioned in the light path of optical signal **106** so as to filter out infrared radiation which may be generated by third housing **146**, when its temperature is rising.

As stated, optical signals generated in different reaction chambers can be spatially separated, for example, using an optical focusing device **112**, so as to prevent cross talks between the different optical signals. Representative examples of device **112** include without limitation, a lens, a plurality of lenses (*e.g.*, a micro-lens matrix) and a video lens. Device **112** can be positioned on second **142** or third housing **146**. Similarly to the above description, device **112** can either focus optical signal **106** on detector **108** or collimate optical signal **106** such that optical signals of different chambers propagate substantially in parallel directions.

Optical focusing device **112** can also be used for further separation of excitation light **100** from optical signal **106**. This can be better understood from Figure 14, which is a simplified illustration of the light path of excitation light **100** once entering chamber **12**. Figure 14 is rotated anticlockwise by 90° relative to Figure 13.

Due to the use of optical fibers **134**, the rays of light **100** are substantially parallel. When light **100** enters chamber **12** it can (i) absorbed by sensors **18** which in response emits optical signal **106**; (ii) scatter off sensors **18** and continue to propagate in a diverted direction; or (iii) continue to propagate in its original direction without interacting sensors **18**. Focusing device **112** is preferably oriented in a manner such that the parallel, non-interacting, light rays are focused by focusing device **112** to its focal point. According to a preferred embodiment of the present invention an opaque object or a reflector **113** can is positioned in the focal point of focusing device **112** so as to absorb or reflect light **100** hence to prevent it from arriving to detector **108**. Reflector **113** is preferably sufficiently small so as not to absorb or reflect off-focal rays. Unlike light **100**, optical signals **106** are emitted and propagated in a plurality of directions, so that only a small portion of optical signals **106** is focused to the focal point of focusing device **112**. Being sufficiently small, the effect of reflector **113** is negligible for optical signals **106**. On the other hand, excitation light rays which are scatter off sensors **18** without being absorbed thereby arrive to focusing device **112** in a direction which may be not parallel to its focal axis. Such non-parallel rays, however, are absorbed by emission filter **110**.

Reference is now made to Figure 15 which illustrates focusing device **112** in the embodiment in which focusing device **112** is a video-lens. The advantage of using a video-lens is that this device is capable of simultaneously projecting optical signals **106** from many reaction chambers to detector **108**, substantially without cross-talks. In addition, a video-lens having a low focal number is capable of collecting a significant part of optical signal **106** with minimal loses.

Reference is now made to Figures 16a-c, which are schematic illustrations of light source **120** in the embodiment of system **20** in which external optical fibers **134** are employed (see Figure 13). Hence, light source **120** preferably comprises a plurality of light emitting devices **122** covered by collimating lens **126** and arranged in a manner that light emitted by each one of devices **122** enters on one or more of optical fibers **134**. For example, a plurality of grooves **136** can be circularly arranged in front of a collimating lens of a single light emitting device, such that light rays having a predetermined impinging angle enter the optical fibers. One of ordinary skill in the art will appreciate that the circular arrangement of grooves **136** ensures that each optical fiber is impinged by the light substantially at the same angle. Referring to Figure 16c, when a plurality of light emitting devices **122** is used, each device can provide excitation light to many optical fibers hence also to many reaction chambers. For example, in the embodiment shown in Figure 16c, there are four light emitting devices, each providing excitation light to nine optical fibers, hence to nine reaction chambers.

Reference is now made to Figures 17a-c which are simplified illustrations of system **20** in the embodiment in which light source **120** is positioned between device **10** and light detector **108**. In this embodiment, system **20** preferably comprises device **10** and an apparatus **160** for imaging the pattern of optical signals **106**. Apparatus **160** comprises detector **108**, an optical element **166** which may be, for example, a plurality of lenses **167** and light source **120**. Lenses **167** are preferably arranged in an arrangement which is compatible with the arrangement of chambers **12** in device **10**, such that each lens is allocated to a predetermined number of chambers (*e.g.*, one lens per chamber).

Referring to Figure 17a, light source **120** can be an arrangement of light emitting devices **122**, which is preferably compatible with the arrangement of lenses **167** such that each light emitting device is allocated to a predetermined number of

lenses (*e.g.*, one light emitting device per lens). Shown in Figure 17a is a rectangular arrangement of light emitting devices **122**, in which the distance between two adjacent light emitting devices is x_1 in one direction (say, the "x" direction) and y_1 in the orthogonal direction (say, the "y" direction). Typical value for both x_1 and y_1 is a few millimeters, for example, 1 millimeter. x_1 can be equal to, or different from y_1, depending on the desired geometrical arrangement, for example, the density of light emitting devices **122** in the respective direction. The transverse size of each light emitting device, designated s in Figure 17a, is typically from about 10 μm to about 20 μm. Light emitting devices **122** can be activated simultaneously or independently.

Figures 17b-c show one light emitting device and one lens respectively designated by numerals **122** and **167**. Detector **108** is preferably connected to a first substrate **162**, which may be, for example, a glass substrate coated so as to prevent randomly reflected excitation rays from penetrating therethrough. In embodiments in which infrared filter **148** is employed, infrared filter **148** is preferably formed on first substrate **162** and detector **108** is connected to infrared filter **148**. Light emitting device **122** is preferably connected to a second substrate **164**, which can be, for example, a sapphire substrate.

According to a preferred embodiment of the present invention light emitting device is configured to generate excitation light **100** in a direction other than a direction of detector **108**. This can be done, for example, by positioning opaque object or reflector **113** adjacently to light emitting device **122**, between light emitting device **122** and detector **108** thereby to prevent light **100** from impinging on detector **108**. Reflector **113** can also have a non planar shape (*e.g.*, parabolic or hyperbolic shape) so as to increase the amount of excitation light propagating in the direction of device **10**.

Figure 17b show the light path of excitation light **100**. As shown, light emitting device **122** is preferably positioned at the focal point of lens **167**, so that excitation light **100** is collimated by lens **167**, and impinges on sensors **18** of device **10** in a form of a collimated beam.

Figure 17c show the light path of optical signals **106**, emitted by sensors **18**. According to a preferred embodiment of the present invention, lens **167** is positioned in a manner such that optical signals **106** are focused by lens **167** to impinge on detector **108**. This can be achieved by positioning lens **167** half way between detector **108** and device **10**, at two focal distances therefrom. Only collimated light rays have a

light path which goes through the focal point of lens **167**. Thus, being emitted at a plurality of directions, a large portion of optical signals **106** arriving at lens **167** is not collimated, and therefore is not affected by reflector **113**.

The above selection of two focal distances between lens **167** and device **10** on the one side, and between lens **167** and detector **108** on the other side, ensures mapping of sensors **18** or chambers **12** on detector **108**. More specifically each one of reaction chamber **12** is represented by an addressable region on detector **108**. When sensors of a particular reaction chamber emit optical signal **106** detector **108** detects this signal at the respective addressable region. Thus an image the emitting sensors is formed on detector **108**. Knowing the reactivity properties of the sensors of the respective reaction chamber in device **10**, the image can be used, for example, by data processor **23** (not shown, see Figure 8c), for determining the presence and/or concentration of the analyte(s) with which the sensors react.

As stated, the sensors which are employed by the present invention are capable of generating a detectable signal when exposed to the at least one analyte in the sample. According to a preferred embodiment of the present invention sensors **18** are biological sensors. Many biological sensors are contemplated. Preferably the biological sensors are made of a biological material (*e.g.*, cell population) capable of producing a material when exposed to the analyte. Representative examples of the produced material include, without limitation, a bioluminescent material, a phosphorescent material and a fluorescent material. Alternatively, the bilogical sensors can produce a material which is capable of altering the electrostatic characteristic of the sample.

Although numerous examples of biological sensors exist in the art, these are limited by instability of the biological component, irreversibility, costs of production and limited ability to identify broad range of analytes.

To overcome such limitations, the present inventors have devised and constructed a reporter-expressing cell population which is composed of discrete subpopulations each capable of expressing the reporter in response to a different analyte or groups of analytes. When exposed to an analyte, the various subpopulations produce a specific expression pattern which forms a signature profile specific to the analyte present in the sample. To enable such analyte specific expression, the present inventors carefully selected a group of promoters which can be activated by different

analytes from a number of promoter libraries. It is postulated herein that by utilizing a broad range of physiologically-responsive promoters, one increases an ability of a cell population transformed with reporter constructs containing such promoters to uniquely respond (via unique reporter expression patterns) to each of a broad range of analytes.

Thus, according to a further aspect of the present invention there is provided a population of cells which can be utilized as biological sensors. The population of cells is composed of at least two subpopulations of cells. A first such subpopulation includes a first reporter expression construct which is capable of reporter expression when the cells of this subpopulation are exposed to a first analyte. A second subpopulation of cells includes a second reporter expression construct which is capable of reporter expression when the cells of the second subpopulation are exposed to a second analyte.

As used herein "population of cells" refers to prokaryotic or eukaryotic cells which can be genetically modified (in a transient or stable manner) to express exogenous polynucleotides.

Examples of prokaryotic cells which can be used in accordance with this embodiment include but are not limited to bacterial cells, such as Pseudomonas, Bacillus, Bacteriodes, Vibrio, Yersinia, Clostridium, Mycobacterium, Mycoplasma, Coryynebacterium, Escherichia, Salmonella, Shigella, Rhodococcus, Methanococcus, Micrococcus, Arthrobacter, Listeria, Klebsiella, Aeromonas, Streptomyces and Xanthomonas.

Examples of eukaryotic cells which can be used in accordance with the present embodiment include but are not limited to cell-lines, primary cultures or permanent cell cultures of fungal cells such as Aspergillus niger and Ustilago maydis [Regenfelder, E. *et al.* (1997) EMBO J. 16:1934-1942], yeast cells (see U.S. Patent Nos. 5,691,188, 5,482,835), such as Saccharomyces, Pichia, Zygosaccharomyces, Trichoderma, Candida, and Hansenula, plant cells, insect cells, nematoda cells such as c. elegans, invertebrate cells, vetebrate cells and mammalian cells such as fibroblasts, epithelial cells, endothelial cells, lymphoid cells, neuronal cells and the like. Cells are commercially available from the American Type Culture Co. (Rockville, Md).

As mentioned hereinabove, the population of cells preferably includes at least two subpopulations of cells. However, it is appreciated that the more subpopulations

included in the cell population the higher the chances of such a cell population to accurately identify analytes present in a sample exposed thereto.

As mentioned hereinabove, each subpopulation of cells includes a reporter expression construct, which expresses a detectable reporter molecule when the cell is exposed to an analyte.

As used herein "reporter expression construct" refers to a vector which includes a polynucleotide sequence encoding a reporter. The reporter expression construct is preferably designed to randomly integrate into the genome of the cell, such that expression of the reporter polypeptide is governed by an endogenous regulatory element which is inducible by an analyte.

According to a preferred embodiment of the present invention, the polynucleotide sequence is positioned in the construct under the transcriptional control of at least one cis-regulatory element suitable for directing transcription in the subpopulation of cells upon exposure to an analyte.

As used herein a "cis acting regulatory element" refers to a naturally occurring or artificial polynucleotide sequence, which binds a trans acting regulator and regulates the transcription of a coding sequence located down-stream thereto. For example, a transcriptional regulatory element can be at least a part of a promoter sequence which is activated by a specific transcriptional regulator or it can be an enhancer which can be adjacent or distant to a promoter sequence and which functions in up regulating the transcription therefrom.

It will be appreciated that the cis-acting regulatory element of the presently preferred embodiment of the invention may be stress regulated (*e.g.*, stress-regulated promoter), which is essentially activated in response to cellular stress produced by exposure of the cell to, for example, chemicals, environmental pollutants, heavy metals, changes in temperature, changes in pH, as well as agents producing oxidative damage, DNA damage, anaerobiosis and changes in nitrate availability or pathogenesis.

Examples of promoters which are preferably used in accordance with the presently preferred embodiment of the invention include, but are not limited to, MipA, LacZ, GrpE, Fiu, MalPQ, oraA, nhoA, recA, otsAB and yciD.

A cis acting regulatory element can also be a translational regulatory sequence element in which case such a sequence can bind a translational regulator, which up regulates translation.

The term "expression" refers to the biosynthesis of a gene product. For example, in the case of the reporter polypeptide, expression involves the transcription of the reporter gene into messenger RNA (mRNA) and the translation of the mRNA into one or more polypeptides.

As used herein "reporter polypeptide" refers to a polypeptide gene product, which, can be quantitated either directly or indirectly. For example, a reporter polypeptide can be an enzyme which when in the presence of a suitable substrate generates chromogenic products. Such enzymes include but are not limited to alkaline phosphatase, β-galactosidase, β-D-glucoronidase (GUS), luciferase and the like. A reporter polypeptide can also be a fluorescer such as the polypeptides belonging to the green fluorescent protein family including the green fluorescent protein, the yellow fluorescent protein, the cyan fluorescent protein and the red fluorescent protein as well as their enhanced derivatives. In such a case, the reporter polypeptide can be quantified via its fluorescence, which is generated upon the application of a suitable excitatory light. Alternatively, a polypeptide label can be an epitope tag, a fairly unique polypeptide sequence to which a specific antibody can bind without substantially cross reacting with other cellular epitopes. Such epitope tags include a Myc tag, a Flag tag, a His tag, a Leucine tag, an IgG tag, a streptavidin tag and the like. Further detail of reporter polypeptides can be found in Misawa et al. (2000) PNAS 97:3062-3066.

It will be appreciated that in certain aspects of the present invention the reporter expression construct may be expressed in response to a growth condition. Examples of such conditions include, but are not limited to temperature, humidity, atmospheric pressure, contact surfaces, radiation exposure (such as, γ-radiation, UV radiation, X-radiation).

As mentioned hereinabove, each reporter expression construct is expressed in a subpopulation of cells upon exposure to a distinct analyte or groups of analytes. It will be appreciated however, that since several unrelated analytes can lead to the same effect on a cell, an expression construct can also be expressed albeit at lower effeciency upon exposure to other analytes. Such partial overlap between the different

reporter expression constructs is desirable since it will increase the detection range of the population to thereby enable identification of numerous analytes even at low concentration levels. For example, if a first analyte induces reporter expression from one subpopulation it may be difficult to distinguish it from a second unrelated analyte which also induces expression in the same subpopulation. However, if several cell subpopulations are induced by a first analyte (each subpopulation expressing a unique level of the reporter) the likelihood that the same subpopulations will also react with the same expression pattern upon exposure to a second analyte is remote.

Dependent on the host cell used, the reporter expression construct can include additional elements. For example, polyadenylation sequences can also be added to the reporter expression construct in order to increase the translation efficiency of a reporter polypeptide expressed from the expression construct of the present embodiment. Two distinct sequence elements are required for accurate and efficient polyadenylation: GU or U rich sequences located downstream from the polyadenylation site and a highly conserved sequence of six nucleotides, AAUAAA, located 11-30 nucleotides upstream. Suitable termination and polyadenylation signals include, without limitation, those derived from SV40.

In addition to the elements already described, the expression construct may typically contain other specialized elements intended to increase the level of expression of cloned nucleic acids or to facilitate the identification of cells that carry the recombinant DNA. For example, a number of animal viruses contain DNA sequences that promote the extra chromosomal replication of the viral genome in permissive cell types. Plasmids bearing these viral replicons are replicated episomally as long as the appropriate factors are provided by genes either carried on the plasmid or with the genome of the host cell.

The construct may or may not include a eukaryotic replicon. If a eukaryotic replicon is present, then the vector is amplifiable in eukaryotic cells using the appropriate selectable marker. If the construct does not comprise a eukaryotic replicon, no episomal amplification is possible. Instead, the recombinant DNA integrates into the genome of the engineered cell, where the promoter directs expression of the desired nucleic acid.

The reporter expression construct can be introduced into the cell using a variety of molecular and biochemical methods known in the art. Examples include,

but are not limited to, transfection, conjugation, electroporation, calcium phosphate-precipitation, direct microinjection, liposome fusion, viral infection and the like. Selection of a suitable introduction method is dependent upon the host cell and the type of construct used.

Since the response of each subpopulation of the cell population of the presently preferred embodiment of the invention to an analyte needs to be assessed independently in order to generate a signature expression pattern, each cell of each subpopulation is preferably tagged with a distinct tag unique to the subpopulation. The tag may be for example, a fluorophoric or chromophoric dye compound which may be detected using a microscope. Such dyes are commercially available such as from Molecular Probes (Eugene, Oregon, USA). Alternatively, cells can be naturally fluorescing or genetically engineered to fluoresce. Molecular tags can also be used. Such tags may be detected by amplification methods, such as PCR.

According to yet another aspect of the present invention there is provided a method of detecting analytes in a sample fluid, the method comprises the following method steps which are illustrated in the flowchart diagram of Figure 18.

In a first step of the method, designated by Block **182** a detecting device, *e.g.*, device **10** is provided. In a second step, designated by Block **184**, a portion or all of the reaction chambers of the detecting device are filled with the sample fluid. The filling can be done by any of the aforementioned transport techniques, *e.g.*, pumping, dielectrophoretic forces, capillary forces, injection and the like. According to a preferred embodiment of the present invention different portions of the reaction chambers of the detecting device can be contain different sensors. For example, when the sensors are cell population, different subpopulations can be placed in different reaction chambers. In addition, several reaction chambers can include only nutritious material for the subpopulations, so as to allow assessment of the contribution of the nutritious material to the detected signals. Still in addition, several reaction chamber may not contain sensors at all, thereby serving as a control group.

According to a preferred embodiment of the present invention different portions of the reaction chambers can be filled with different fluids, in any combination with the different sensors, so as to allow each sensor to be exposed to a plurality of fluids and each fluid to be sensed by a plurality of sensors. This

embodiment is particularly useful for conducting complicated assays, as further detailed hereinunder and exemplified in the Examples section that follows.

In a third step of the method, designated by Block **186**, the sensors generate the detectable signal. This can be done, for example, in response to irradiation of the device by excitation light, as further detailed hereinabove. In a fourth step, designated by Block **188**, the signal is detected, for example, using a planar detector (*e.g.*, detector **108**).

According to a preferred embodiment of the present invention the method further comprises maintaining continues flow of the sample fluid in the channels so as continuously replace the sample fluid the reaction chambers. This step is designated by Block **192** in Figure 18. To facilitate continuous flow, the device preferably comprises a draining system (*e.g.*, output buffer **32**, see Figure 2c) for discarding excess fluids therefrom. Continues flow of sample fluid is advantageous for online monitoring and detection of the sample fluid. Thus, according to a preferred embodiment of the present invention the detection of the generated signals is done substantially in real time.

In an online measurement, it is often desired to have an indication of the general state of the detecting device. The electronic circuitries of the device (*e.g.*, the aforementioned circuitries for controlling the transport mechanism, temperature, light source, detector, *etc.*) can be monitored substantially in real time by incorporating appropriate feedback lines therein. In addition, several sensors of the preferably generate a reference signal at all times, so as to provide indication of their operation. This embodiment is represented in Figure 18 by Block **190**.

When the sensors of the detecting device are biological sensors (*e.g.*, live cells, such as, but not limited to, the aforementioned reporter-expressing cell population), the viability thereof can be monitored by incorporating in a few reaction chambers, a material (*e.g.*, a biological material) which generate a detectable reference signal at all times. The reference signal can be optical, electrical electrochemical or any other signal.

The present embodiment has several advantages. First, the reference signal indicates viability of the detecting device. For example, lack of reference signal can indicate that the cell is dead or significantly damaged.

Second, when the sample is highly toxic, the biological sensors may fail to produce the detectable signal, for example, when the biological sensors are killed by toxic substances in the sample. On the other hand, highly toxic sample may also kill the biological material generating the reference signal. Thus, in this case a cessation of the reference signal indicates a highly toxic sample.

Third, the reference signal can serve as a sensor for abnormal state of the sample. For example, when the reference signal is generated by live cells, an abnormal sample state (*e.g.*, abnormal temperature, abnormal pH level, etc.) may cause a stress to the live cells resulting in a decrease of their ability to generate normal reference signal. A reference signal which is below a predetermined level therefore indicates an abnormal sample state.

Thus, the combination of the reference signal and the sensors of the device allows an efficient detection of the analyte in the sample. According to a preferred embodiment of the present invention, the presence, concentration and/or type of the analyte can be determined. More specifically, the presence of analytes in the sample can be determined by detecting a change in signal reading or a change the rate of change of signal readings (both signal in response to the analyte and reference signals); the concentration of the analyte in the sample is determined by the absolute value of the detected signal; and the type of analyte is determined from the imaging information provided by the detector.

As stated, different portions of the reaction chambers of the detecting device can be contain different sensors and/or be filled with different fluids, in any combination with the different sensors. For example, in the embodiment in which the reaction chambers and the fluid channels are arranged in sequential arrays (see Figures 2a-b), each array can be allocated for a different sample fluid, while each reaction chamber in a particular array contain a different sensor. Thus, when a particular sample fluid is transported into the chambers of its respective array, different signals are generated in different reaction chambers, thus enable multiplexing.

Reference is now made to Figure 19, which is a schematic illustration of a logical and physical division of the detecting device, according to the presently preferred embodiment of the invention. As show in Figure 19, the detecting device can be divided into rows and columns. Figure 19 exemplify four columns, designated **194a**, **194b**, **194c** and **194d**, and three rows, designated **196a**, **196b** and **196c**. The

reaction chambers of each column can be in fluid communication thereamongst, so as to allow filling al the reaction chambers of a particular column with the same sample fluid.

The columns can be physically divided so as to prevent sample fluids from flowing across a row of reaction chambers. Each row can be allocated to a different functionality. For example, row **196a** can be allocated for signal stability testing, row **196b** can be allocated for detecting unknown analytes and row **198c** can be allocated for detecting known analytes.

Different columns can be allocated for different sample fluid or the same sample fluid as desired. For example, prior to the transport of the sample fluid into one column, the sample fluid can be purified, *e.g.*, using an activated filter, thus to serve as a control to another column in which no purification was employed.

According to still another aspect of the present invention there is provided a method of dehydrating a biological material. The method can be used for example, when for placing biological sensors, *e.g.*, the aforementioned cell population, in the reaction chambers of device **10**, in a vacuum preservation manner. It is appreciated that when the cell population is dehydrated and kept in vacuum, the cell population can be in dormant state for a prolonged period of time, until device **10** becomes operative.

The method according to the presently preferred embodiment of the invention comprises the following method steps which are illustrated in Figures 20a-c. In a first step of the method, illustrated in Figure 20a, a first set **242** and a second set **244** of chambers are provided. First set **242** preferably contains a biological material **246** and second set **244** preferably has one or more fluid channel **248** formed therein or attached thereto. It is to be understood that although each of first set **242** and second set **244** are represented in Figure 20a-c a single chamber, any number of chambers can be used, in any arrangement, depending on the application for which biological material **246** is employed. For example, when the biological material **246** is to be used as a biological sensor in device **10**, chambers **242** can be lower parts **29** of reaction chambers **12**, and chambers **244** can be upper parts **31** thereof (see Figure 2b). Thus, chamber **244** is preferably designed sizewise and shapewise compatible with chamber **242** so as to allow covering of chamber **242** with chamber **244**. To facilitate the contact between chamber **242** and chamber **244**, and the sealing of chamber **12** once

chambers **242** and **244** are assembled together, an additional layer **243** may be placed between chambers **242** and **243**. Additional layer **243** is preferably made of a rubbery material or any other material having sealing properties.

According to a preferred embodiment of the present invention biological material **246** is immobilized onto chamber **242**. This can be done, for example, by encapsulating biological material **246** into a meltable membrane or a matrix made of, *e.g.*, agar, alginate, poly-vinyl alcohol, sol-gel, carraginan and the like.

In a second step of the method, illustrated in Figure 20b, chambers **242** and **244** are placed in a negative pressure environment **250**, so as to dehydrate biological material **246**. According to a preferred embodiment of the present invention chamber **244** comprises a window **252**, so that when chamber **244** partially covers chamber **242**, evaporation of liquids is allowed through window **252**.

In a third set of the method, illustrated in Figure 20c, chamber **244** are positioned on chamber **242** in a manner such that chamber **242** is sealed. This can be done by pressing mechanical chamber **244** in the direction of chamber **242** until window **252** is completely sealed by walls **254** of chamber **242**. According to a preferred embodiment of the present invention the sealing of window **252** is done in a manner such that fluid channel **248** is not obstructed. The sealing of chamber **242** is preferably executed while both chamber **242** and chamber **244** are in environment **250** so as to maintain negative pressure therein. Once chamber **242** is sealed by chamber **244** they can be removed from environment **250**. However, the internal negative pressure of chambers **242** and **244** is preferably maintained, so as to prevent humidity and gasses (*e.g.*, air, oxygen) from penetrating into chamber **12**. The internal negative pressure is particularly useful when the present method is used for manufacturing device **10** for the purpose of future use. In such cases, it is desired to have the biological sensors in an inactive state while device **10** is not being used. One ordinarily skilled in the art would appreciate that as long as humidity and gasses are prevented from penetrating the chamber, the biological sensors remain inactive. Only once the sample fluid is transported through channel **248**, the desired biological activity is initiated.

Thus, the present invention successfully provides a method of placing biological sensors in device **10**, in a manner such that (i) negative pressure is maintain so that the biological sensors are in a dormant state until device **10** is operative; and

(ii) the fluid channels are not obstructed so that when device **10** becomes operative, the sample fluid can be transported to the reaction chambers through the fluid channels as further detailed hereinabove. When the sample fluid is transported into device **10**, the meltable membrane is melted thereby facilitating interaction between the biological sensor and the sample fluid. As a result of the interaction, the biological sensor produces a material generating the detectable signals. As stated, the produced material can be a fluorescent material so that optical signals are generated thereby.

It is appreciated that when the sensors are biological sensors, each reaction chamber may contains many biological reporters, each located in a different location in the reaction chamber, hence contributes differently to the overall detected optical signal. The ability to accurately determine the concentration of the analyte therefore depends on a judicious calculation of the different contributions thereto. The present invention successfully provides a method of determining the concentration of the analyte in the fluid sample, from the optical signals generated by the fluorescent material. The method is based on a slicing technique and comprises the following method steps which are illustrated in the flowchart diagram of Figure 21.

Hence, in a first step, designated by block **302**, a plurality of slices is defined, where each slice includes at least one biological reporter. Preferably, the definition of slices is done for each of the reaction chamber of device **10**. Ideally, each slice is two dimensional, however, although not excluded, such slices are hardly attainable. Thus, according to a preferred embodiment of the present invention the slices are defined such that the thickness of each slice is small, preferably about one third, more preferably one tenth, most preferably one hundredth or one thousandth of the depth of the reaction chamber. A representative example of calculating the thickness of a slice is given in the Examples section that follows (see Example 2).

In the second step of the method, designated by Block **304**, all the biological reporters of a slice are represented as at least one equivalent light emitter, located at a predetermined location within the slice, for example, at the center of the slice. For each slice, the respective equivalent light emitter imitates all the biological reporters in the slice. In other words, the detected radiation is composed of a plurality of local radiation contributions, each emitted by one of the equivalent light emitters. The calculation of the local radiation contribution of each equivalent light emitter preferably comprises calculation of effective quantum efficiency preferably both for

emission and for excitation, and at least one transmission coefficient corresponding to the excitation light and the light emitted by the equivalent light emitter.

In a third step of the method, designated by Block **306**, the local radiation contribution is integrated over all or most of the slices of each reaction chamber so as to obtain an integrated radiation intensity. The integration in this step is preferably done along an axes perpendicular to the plane of each slice.

In the forth step of the method, designated by Block **308**, the recorded optical signals and the integrated radiation intensity are used for determining the concentration of the analyte. This can be done by calculating an occupation area of the fluorescent material, in each reaction chamber. The occupation area is preferably defined as a projection of an occupation volume on a plane perpendicular to a direction of the excitation light. The occupation volume depends on the intensity of radiation emitted by fluorescent material. Thus, according to a preferred embodiment of the present invention the occupation volume is calculated by tracing the light rays emitted by the equivalent light emitters using optical geometry techniques, as further detailed in the Examples section that follows (see Example 2).

Additional objects, advantages and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions illustrate the invention in a non limiting fashion.

EXAMPLE 1

CMOS Detector

Reference is now made to Figure 22a-b exemplifying an electronic diagram of a CMOS detector **200**, which can be used as detector **108**, according to a preferred

embodiment of the present invention. CMOS detector **200** is known in the art and can be purchased, for example, from Fill Factory, Mechelen Belgium.

CMOS detector **200** comprises a matrix **201** of elementary units **202** referred to herein as pixels **202**. Figure 22b shows one pixel **202**, which comprises a capacitor **204** which is pre-charged to a reset bias voltage, a photodiode **206,** for discharging capacitor **204** in response to photons absorption and 3 MOS transistors, designated **208a, 208b** and **208c,** for resetting (transistor **208a**), sensing (transistor **208b**) and leading (transistor **208c**) the signal to column amplifier **210**.

CMOS detector **200** further comprising a left vertical shift register **212** and a right vertical shift register **214**. Left register **212** serves a pointer to a row that is pre-charged to reset bias voltage (reset row operation) and right register **214** serves as a pointer to a row which is being read by column amplifier **210**. The distance between two pointers determines the integration time, T_{int}, which can be calculated as follows:

$$T_{\text{int}} = N_{\text{int}-rows} \cdot \left(T_{rd-px} \cdot N_X + T_{blanking}\right), \qquad \text{(EQ. 1)}$$

where $N_{int\text{-}rows}$ is the distance in rows between the readout and the reset row pointers, $T_{rd\text{-}px}$ is the time for one pixel readout, N_X is the size of the window in X-direction and the $T_{blanking}$ is the minimal required time between two successive row readouts. $T_{rd\text{-}px}$ and $T_{blanking}$ depend on signals generated in a clock unit **222**.

CMOS detector **200** further comprises a column shift register **216** which selects the appropriate column amplifier **210**. The signal from column amplifier **210** is transmitted through a pre-amplifier **218** to a digital converter **220**.

Pre-amplifier **218** and converter **220** preferably operate in linear mode. The quantified output of CMOS detector **200** is proportional to the number of photons colliding on pixels **202**, denoted N_{pht}:

$$N_{pht}(T_{\text{int}}) = C\frac{\left(QO(T_{\text{int}}) - QO_{DC\_e\text{-}}(T_{\text{int}})\right)/2^{bits}}{QE \cdot FF}, \qquad \text{(EQ. 2)}$$

where QO is a quantified output, *bits* is the quantization of converter **220**, QE is a quantum efficiency of the CMOS detector **200**, FF is a fill-factor, C is a maximal capacity of pixel in photo-electrons and $QO_{DC\_e\text{-}}$ is a quantified output of an integrated dark current, I_{DC}.

The time dependence of $QO_{DC\_e\text{-}}$ is given by:

$$QO_{DC\_e-}(T) = \frac{I_{DC}}{C} T \cdot 2^{bits}, \qquad \text{(EQ.3)}$$

where the units of I_{DC}, C and T are such that the expression $I_{DC}T/C$ is dimensionless. For example, I_{DC}, C and T can be measured in e^-/s, e^- and s, respectively.

For the CMOS detector of the present example, a typical value of C, is 6×10^4. The value of the dark current, I_{DC}, is about 1055[e^-/s], for a temperature of 21 °C.

The required integration time is inversely proportional to the intensity of the signals. On the other hand, this maximal integration time is limited because of dark current saturation effect.

For weak signals, the maximal integration time is given by:

$$T_{\text{int-max-}ws} = \frac{C}{I_{DC}} \cdot F_{DC} \qquad \text{(EQ. 4)}$$

where F_{DC} is a parameter defined by the following equation:

$$QO_{DC\_e-\_\max} = F_{DC} \cdot 2^{bits}. \qquad \text{(EQ. 5)}$$

A typical value of F_{DC} is about 0.5.

For strong signals, the maximal integration time is given by:

$$T_{\text{int-max-}ss} = \frac{C}{I_s \cdot QE \cdot FF}, \qquad \text{(EQ. 6)}$$

where I_s is the strong signal current.

EXAMPLE 2

Determination of Analyte Concentration Using the Detected Signals

Reference is now made to Figures 23a-c, which illustrate the radiation emitted by one reaction chamber of device **10**.

Figure 23a illustrates reaction chamber **12**, a plurality of locations **320**, where biological cells generating the florescent materials are located, and a slice **322** defined in accordance with a preferred embodiment of the present invention. Reaction chamber **12** has an aperture **326** through which optical signals **106** (not shown, see Figures 23b-c) exit. In the following calculations, slice **322** is represented as equivalent light emitter **324**, shown in Figure 23b. Equivalent light emitter **324** is a superposition of the all the light emitter in slice **322** and can be defined, for example, by integration or summation. Also shown is excitation light **100** and optical signal **106** emitted in a plurality of directions.

Figure 23c illustrates the spreading of optical signal **106** through aperture **326** of the reaction chamber. The respective numerical aperture for optical signal **106**, designated herein by β'_{Ω}, depends on the position of slice **322**. Optical signal **106** is collimated by lens **112** prior to impingement of optical signal **106** on light detector **108**. Being spaced apart from aperture **326** the corresponding numerical aperture of lens **112**, designated herein by α'_{Ω}, is smaller than the numerical aperture of aperture **326**, β'_{Ω}. Similarly to β'_{Ω}, α'_{Ω} also depends on the position of slice **322**. Emission filter **110** is positioned in the light path of optical signal **106** so as to prevent rays of excitation light **100** from arriving to detector **108**. Emission filter **110** preferably allows transmission of optical signal **106** substantially without loses.

The use of lens **112** is preferred, but optional. In an alternative embodiment in which lens **112** is not used, emission filter **110** and detector **108** are preferably positioned instead of lens **112**, with no substantially change in the values of the β'_{Ω} and α'_{Ω}.

According to a preferred embodiment of the present invention several optical coefficients are calculated. A first optical coefficient is the emission quantum efficiency, denoted herein by *EmQE*, which is the ratio between the absorbed excitation radiation and the emitted radiation. A second optical coefficient is the transmission coefficient of emission filter **110** for excitation light **100**, designated herein by $T_{Fem4exc}$. A third optical coefficient is the transmission coefficient of emission filter **110** for optical signal **106**, designated herein by $T_{Fem4ems}$. A fourth optical parameter is the effective quantum efficiency of detector **108** for optical signal **106**, designated herein by QE_{ems}, and a fifth optical parameter is the effective quantum efficiency of detector **108** for excitation light **100**, designated QE_{exc}.

The value of the above five coefficients depends on the spectral characteristics of the optical components. More specifically, the following spectra are used for the calculations: (i) the spectrum of the light source, S_L; (ii) the spectrum of excitation filter S_{Fexc} (iv) The spectrum of emission filter **110**, S_{Fem}; (v) the efficiency of the fluorescence excitation E_{fl}; (vi) the emission spectrum, S_{ems}; and (vii) the Quantum Efficiency (QE) of light detector **108**.

The calculation of the optical coefficient can be done using the following formulae:

$$EmQE = \frac{\sum_{\lambda} S_L S_{Fexc} E_{fl}}{\sum_{\lambda} S_L S_{Fexc}}, \quad \text{(EQ. 7a)}$$

$$T_{Fem4exc} = \frac{\sum_{\lambda} S_L S_{Fexc} S_{Fems}}{\sum_{\lambda} S_L S_{Fexc}}, \quad \text{(EQ. 7b)}$$

$$T_{Fem4ems} = \frac{\sum_{\lambda} S_{ems} S_{Fems}}{\sum_{\lambda} S_{ems}}, \quad \text{(EQ. 7c)}$$

$$QE_{ems} = \frac{\sum_{\lambda} S_{ems} S_{Fems} QE}{\sum_{\lambda} S_{ems} S_{Fems}}, \quad \text{(EQ. 7d)}$$

$$QE_{exc} = \frac{\sum_{\lambda} S_L S_{Fexc} S_{Fems} QE}{\sum_{\lambda} S_L S_{Fexc} S_{Fems}}, \quad \text{(EQ. 7e)}$$

where A_{bsrp} is the ratio between the area of equivalent light emitter **324** to the area of the excitation beam, n_{GFP}, is the occupation of the fluorescent material, measured as a percentage of the area of equivalent light emitter **324**, $N_{\text{ems-ph}}$ is a number of the emission photons per second, $N_{\text{exc-ph}}$ is a number of the excitation photons per second, $E_{\text{ph-470}}$ is energy of single photon at the wavelength 470nm and P_{exc} is the optical power. Figure 24 is a schematic calculation diagram which can be implemented for the calculation of Equations 7a-7e.

According to a preferred embodiment of the present invention once the optical coefficients are calculated, a ray tracing procedure is employed.

Figure 25 illustrates light propagation from equivalent light emitter **324** to lens **122**. Light rays are redirected to the angle β'_{Ω}, according to Snell's law

$$n_{RC} \cdot \sin(\beta_{\Omega}/2) = n \cdot \sin(\beta'_{\Omega}/2) \quad \text{(EQ. 8)}$$

where n_{RC} is the refraction index of the medium in reaction chamber **12** and n is the refraction index of the external medium.

Assuming that the there are B_{cube} biological reporters in a 1mm^3 cube, the number of biological reporters in one dimension B_{1D}, is the cubic root of B_{cube}. Thus, denoting the height of reaction chamber by H, the total number of slices is:

$$N_{layers} = B_{1D-1mm} \cdot (H/1mm), \quad \text{(EQ. 9)}$$

Thus, each slice has a thickness of:

$$\Delta h = H/N_{layers}, \quad \text{(EQ. 10)}$$

and is centered at position H_{2i}, where:

$$H_{2i} = H - \Delta h \cdot (i - 0.5). \quad \text{(EQ. 11)}$$

The scattering angle of optical signal from the *ith* slice is given by:

$$\beta_{\Omega i} = 2 \cdot tg^{-1}(W/2H_{2i}), \quad \text{(EQ. 12)}$$

where W is the diameter of aperture **326**.

Using Equations 8 and 12 one can calculate the numerical aperture, β'_{Ω}:

$$\beta'_{\Omega} = 2 \cdot \sin^{-1}\left((n_{RC}/n) \cdot \sin\left(tg^{-1}(W/2H_{2i})\right)\right). \quad \text{(EQ. 13)}$$

The numerical aperture of the lens for the *i*th slice is given by:

$$\alpha_{\Omega i} = 2 \cdot tg^{-1}(d1/2H2_i), \quad \text{(EQ. 14)}$$

where H_{2i} is the distance between the center of the ith slice and output aperture and *d*1 is twice the distance between the optical axis of the lens and the emitted light ray (see Figure 25), which can be numerically calculated, for example, by an iterative procedure.

Figure 26 illustrates the scattering solid angle of the emitted light rays. Hence, defining the range $\alpha\beta_{\Omega i}$ as $\min(\alpha_{\Omega i}, \beta_{\Omega i})$, light rays emitted from the *i*th slice at an angle within range $\alpha\beta_{\Omega i}$, are redirected to detector **108**.

Generally, rays **106** are scattered uniformly to a solid angle of 4π. The fraction of emission energy impinging on detector **108** is therefore:

$$F_{GFP} = \frac{\Omega_{\alpha\beta i}}{4\pi}, \quad \text{(EQ. 14)}$$

where $\Omega_{\alpha\beta i}$ is the solid angle corresponding to range $\alpha\beta_{\Omega i}$ and is given by:

$$\Omega_{\alpha\beta i} = 2\pi(1 - \cos(0.5\alpha\beta_{\Omega i})). \quad \text{(EQ. 15)}$$

Following is a description of a calculation of the absorption of the excitation light and the corresponding emission of optical signals **106**.

A photon having wavelength λ carries energy which equals:

$$E_{ph}(\lambda) = \frac{h \cdot c}{\lambda}. \quad \text{(EQ. 16)}$$

For example, when the excitation wavelength is 470 nm, the energy carried by one

excitation photon is 3.810^{-19} J. Assuming that the excitation light is transmitted by an optical fiber, photon flux (the number of photon per unit time) is given by:

$$I_{exc} = \frac{P_{fiber}}{E_{ph}(\lambda_{exc})}, \quad \text{(EQ. 17)}$$

where P_{fiber} is the optical power of the optical fiber which can be measured, for example, using an optical power meter positioned on the output of the optical fiber.

The amount of optical signals generated by the biological material is proportional to the projection area of the fluorescent material on a plane perpendicular to the direction defined by detector **108** and slice **324**.

For a $d_b \times d_b \times d_b$ cube, the maximum absorption by the fluorescent material of the *i*th slice is:

$$A_{GFP-\max-i} = \frac{d_b^2 \cdot B_{layer}}{\pi \cdot (W/2)^2}, \quad \text{(EQ. 18)}$$

where B_{layer} is the number biological reporters in *i*th slice:

$$B_{layer} = \frac{B_{RC}}{N_{layers}}. \quad \text{(EQ. 19)}$$

The maximal absorption occurs for when the biological material is completely saturated by the fluorescent material. The efficiency of the bio-chemical reaction is proportional to the percentage of the biosensor saturation by the GFP molecules. The percentage of the saturation in layer *i* is signed as n_{GFP-i}.

The light absorption in the *i*th layer is given by:

$$A_{GFP-i} = A_{GFP-\max-i} \cdot n_{GFP}, \quad \text{(EQ. 20)}$$

where $A_{GFP-max-i}$ is the maximal absorption.

The value of the n_{GFP} can be between zero and unity inclusive. $n_{GFP} = 0$ means that is that no fluorescent material was generated, while $n_{GFP} = 1$ means that the entire biological cell is saturated by fluorescent material and the absorption equals $A_{GFP-max-i}$.

Assuming that the dominant attenuation of the excitation light is due to the fluid in the reaction chamber, the absorbed excitation light in the *i*th slice is given by:

$$I_{exc-i} = I_{exc-i-1} \cdot T_{exc}(\Delta h) = I_{exc} \cdot T_{exc}^{\,i-1}(\Delta h). \quad \text{(EQ. 21)}$$

The maximal intensity of the emitted optical signal, scattered at a solid angle of 4π, can be written as:

$$I_{ems-\max-i} = A_{GFP-\max-i} \cdot I_{exc-i} \cdot EmQE, \qquad \text{(EQ. 22)}$$

and the optical signal intensity as function of the bio-chemical reaction efficiency is therefore given by:

$$I_{ems-i} = I_{ems-\max-i} \cdot n_{GFP}. \qquad \text{(EQ. 23)}$$

Integrating over all slices one thus obtain a relation between the detected signal and n_{GFP}. One ordinarily skilled in the art would appreciate that from the value of n_{GFP} the concentration of the analyte can be obtained, for example, using a simple calibration curve.

EXAMPLE 3

Sensitivity Calculation

As stated, the emission intensity is proportional to the biochemical reaction percentage expressed by the n_{GFP} parameter. In the present example, a sensitivity calculation is performed using a signal uncertainty parameter, which is proportional to the unfiltered excitation intensity detected by the light detector:

$$I_{unc-px} = Un2B \cdot I_{exc-px} \cdot QE_{exc}, \qquad \text{(EQ. 24)}$$

where $I_{\text{unc-px}}$ is the signal uncertainty parameter, $Un2B$ is the ratio between the uncertainty to the background radiation, $I_{\text{ems-px}}$ is the unfiltered excitation intensity as detected by the light detector and QE_{exc} is, as stated, the effective quantum efficiency of the detector for excitation light. A tipical value for $Un2B$ is about 0.5.

The minimal sensitivity is preferably defined such that the sensed optical signal is at least $S2Un$ times stronger that the signal uncertainty, where $S2Un$ is the ratio between the signal to the uncertainty:

$$I_{ems-px} \cdot QE_{ems} \geq S2Un \cdot I_{unc-px}, \qquad \text{(EQ. 25)}$$

where $I_{\text{ems-px}}$ is the emission intensity as detected by the light detector, and QE_{exc} is the effective quantum efficiency of the detector for emission light. The emission intensity from the biological sensor is proportional to the n_{GFP}, thus:

$$R_{ems/exc} = R_{ems/exc-\max} \cdot n_{GFP}$$

$$I_{ems-px} = I_{ems-\max-px} \cdot n_{GFP}, \qquad \text{(EQ.26)}$$

where, $R_{\text{ems/ex}}$ is the ratio between emission and excitation, $R_{\text{ems/ex-max}}$ is the maximal ratio between emission and excitation and $I_{\text{ems-px}}$ is the maximal emission intensity which can be detected.

Combining the Equations 24-26 one has:

$$\frac{I_{ems-\max-px}\cdot QE_{ems}}{I_{exc-px}\cdot QE_{exc}}\cdot n_{GFP}\geq S2Un\cdot Un2B, \qquad \text{(EQ. 27)}$$

Denoting:

$$R_{ems/exc-\max}=\frac{I_{ems-\max-px}\cdot QE_{ems}}{I_{exc-px}\cdot QE_{exc}}, \qquad \text{(EQ. 28)}$$

Equation 27 can be written as:

$$R_{ems/exc-\max}\cdot n_{GFP}\geq S2Un\cdot Un2B. \qquad \text{(EQ. 29)}$$

The constant $R_{ems/exc-max}$ depends on the optical setup, and can be determined, experimentally, or by geometrical optic calculations. From Equation 29 it follows that the minimal optical system sensitivity to the bio-chemical reaction is:

$$n_{GFP-\min}=\frac{S2Un\cdot Un2B}{R_{ems/exc-\max}}. \qquad \text{(EQ. 30)}$$

EXAMPLE 4

Biological Signal Quantification

In this example, the biological signal quantification is demonstrated for a system in which excitation energy is transmitted using external optical fibers and the sensors are E.coli bacteria, capable of producing GFP molecules when interacting with a toxic material. The bacteria were placed in the reaction chamber in the presence of a Luria Bertani nutrition medium.

The detected signal, $QO_{int-sig}$, is a sum of the following contributions (i) unfiltered excitation intensity, $I_{exc-Fem4exc}$; (ii) intensity of emission generated by nutrient medium, I_{LB},; (iii) intensity of light emitted by the GFP molecules, I_{GFP}; and (iv) dark current contribution of the light detector, I_{DC}.

The overall emission intensity that reaches the pixels of the detector is given by:

$$I_{ems-mlens-\det}=\sum_{i=1}^{i=N_{layers}}\left(A_{GFP-\max}\cdot I_{exc-i}\cdot n_{GFP}\cdot T_{ems}(H_{2i})\cdot\frac{\Omega_{\alpha\beta i}}{4\pi}\cdot T_{mlens}\cdot T_{Fems4ems}\right) \qquad \text{(EQ.31)}$$

For an excitation wavelength of 470 nm, the excitation at the *i*th slice is:

$$I_{exc-i} = \left(\frac{P_{fiber}}{E_{ph}(470nm)}\right) \cdot T_{exc}{}^{i-1}(\Delta h) = P_{fiber} \cdot \left(\frac{T_{exc}{}^{i-1}(\Delta h)}{E_{ph}(470nm)}\right). \quad \text{(EQ. 32)}$$

The light intensity, emitted by the GFP molecules is therefore given by:

$$I_{ems-mlens-\det} =$$

$$= \sum_{i=1}^{i=N_{layers}} \left(A_{GFP-\max} \cdot P_{fiber} \cdot \left(\frac{T_{exc}{}^{i-1}(\Delta h)}{E_{ph}(470nm)}\right) \cdot n_{GFP} \cdot T_{ems}(H_{2i}) \cdot \frac{\Omega_{\alpha\beta i}}{4\pi} \cdot T_{mlens} \cdot T_{Fems4ems} \right) =$$

$$= n_{GFP} \cdot P_{fiber} \cdot \underbrace{\sum_{i=1}^{i=N_{layers}} \left(A_{GFP-\max} \cdot \left(\frac{T_{exc}{}^{i-1}(\Delta h)}{E_{ph}(470nm)}\right) \cdot T_{ems}(H_{2i}) \cdot \frac{\Omega_{\alpha\beta i}}{4\pi} \cdot T_{mlens} \cdot T_{Fems4ems} \right)}_{K_{bs-exc-ems-optics-mlens}}$$

(EQ. 33)

where $K_{bs\text{-}exc\text{-}ems\text{-}optics\text{-}mlens}$ is a constant coefficient depending on the geometry of the reaction chamber, the concentration of bacteria, the optical characteristics of the optical components (filters and lenses) and the photon energy. The emission intensity which achieves the light detector is given by:

$$I_{ems-mlens-\det} = n_{GFP} \cdot P_{fiber} \cdot K_{bs-exc-ems-optics-mlens} \cdot \quad \text{(EQ. 34)}$$

Denoting the area onto which the optical signal is spread on the detector by $S_{ems\text{-}det}$, and the area of one pixel by S_{px}, the emission intensity projected on one pixel is:

$$I_{ems-mlens-px} = n_{GFP} \cdot P_{fiber} \cdot K_{bs-exc-ems-optics-mlens} \cdot \left(S_{px}/S_{ems-\det}\right) = n_{GFP} \cdot P_{fiber} \cdot K_{bs-px}$$

(EQ. 35)

where, $K_{bs\text{-}px}$ is a numerical coefficient which can be written as:

$$K_{bs-px} = \frac{S_{px}}{S_{ems-\det}} \cdot \sum_{i=1}^{i=N_{layers}} \left(A_{GFP-\max} \cdot \left(\frac{T_{exc}{}^{i-1}(\Delta h)}{E_{ph}(470nm)}\right) \cdot T_{ems}(H_{2i}) \cdot \frac{\Omega_{\alpha\beta i}}{4\pi} \cdot T_{mlens} \cdot T_{Fems4ems} \right)$$

(EQ. 36)

The relation between the quantified output (QO) of the light detector and the number of photons is given by:

$$N_{pht}(T_{\text{int}}) = C \frac{\left(QO(T_{\text{int}}) - QO_{DC\_e\text{-}}(T_{\text{int}})\right)/2^{bits}}{QE \cdot FF}, \quad \text{(EQ. 37)}$$

where the quantified output due to dark current, QO_{DC}, can be measured by switching off the excitation light source.

Denoting the quantified GFP molecules emission by QI_{GFP}, the occupation of the fluorescent material can be written as:

$$n_{GFP} = QI_{GFP} \cdot \left(\frac{1}{T_{\text{int}} \cdot P_{fiber}} \right) \cdot \left(\frac{1}{K_{bs-px}} \cdot \frac{\left(6e4/2^{bits}\right)}{QE \cdot FF} \right) \qquad \text{(EQ. 38)}$$

The separation of QI_{GFP} from $QO_{int\text{-}sig}$, can be done by logical division of the reaction chambers, in which in a first reaction chamber only the nutrition medium is placed (without the bacteria), hence generates only the contribution of $I_{exc\text{-}Fem4exc}$, I_{LB} and, I_{DC}. In a second reaction chamber both the nutrition medium and the bacteria are present hence generates, once interacting with the fluid sample, all the aforementioned contributions (i)-(iv).

The quantified emission intensity of the GFP molecules is thus obtained by subtraction:

$$QI_{GFP} = QO_2 - QO_1, \qquad \text{(EQ. 39)}$$

where QO_1 and QO_1 are, respectively, the quantified output received from the first reaction chamber (without the bacteria) and the second reaction chamber (with the bacteria).

EXAMPLE 5

Experimental

A prototype system was built according to the teaching of preferred embodiments of the invention described above. The prototype system included a detecting device with 12 reaction chambers and a plurality of micro-pumps were employed (see Figure 3a), and a CMOS light detector (see Example 1). The sensors were E.coli-REC-A bacteria, referred to in this Example as biosensors.

The reaction chamber of the device were used as follows: (2 repetitions) × (2 biosensors *E.coli* – rec-A quantities: 1.5×10^6, 1.5×10^5 [bacteria/1uL]) × (3 Nalidixic Acid concentrations: 0, 5, 10 parts per million. The experiment duration was 5 hours.

Figures 27a-d show the detected optical signal as a function of time. Figures 27a and 27c show the detected optical signal for a concentration of 1.5×10^6 cells per reaction chamber (first and second repetitions, respectively), and Figures 27b and 27d show the detected optical signal for a concentration of 1.5×10^5 cells per reaction chamber (first and second repetitions, respectively).

As shown in Figures 27a-d the strongest emission was registered for $.5\times10^5$ cells per reaction chamber. A significant biochemical reaction was acquired for 5ppm and 10ppm NA.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims. All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention.